

RELATIVE BIOAVAILABILITY OF DIFFERENT
ORGANIC AND INORGANIC ZINC AND COPPER
SOURCES IN RUMINANTS AND RATS

By

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To God.

To my parents, Aquiles J. and Teresita and my brother,
Aquiles.

To my future wife, Marcia M.

To all future graduate students so they also get the
chance I got.

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Chairperson: Dr. L. R. McDowell
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Four experiments (EXP) were conducted to compare the bioavailability (BAV) of amino acid complexed (Zn lysine, ZnLys; Cu lysine, CuLys; Zn methionine, ZnMet) and inorganic sources of Zn and Cu by determining Zn, Cu and metallothionein (MT) concentrations of various fluids and tissues. In EXP 1, cattle were given supplemental (SUP) ZnMet, ZnSO₄, and ZnO (360 mg Zn/d) for 4 wks, withdrawn for 4 wks and resumed for another 4 wks. No treatment (T) differences were determined under those conditions for Zn and Cu in fluids and tissues, and MT in tissues. In EXP 2, wethers were given SUP ZnMet, ZnLys, ZnSO₄, and ZnO (360 mg Zn/kg) for 3 wks, withdrawn for 4 wks and resumed for another wk. By d 49 serum Zn had increased less for controls than most T, and by d 55 had increased more for ZnLys than most T. The ZnLys T had the highest Zn and MT in kidney, liver and pancreas. Both ZnSO₄

and ZnMet had higher liver Zn than controls. Muscle Cu was highest for controls. For EXP 2, organic Zn sources were equally or more BAV than the best inorganic source. In EXP 3, four sheep were administered 250 mg of SUP Cu from either CuLys or CuSO₄. One of the CuSO₄ treated sheep was very sensitive to Cu and the other was not affected by Cu excess. Myodegradation was present in one animal from each T prior to death. For EXP 3, the development of toxicity was not affected by source. In EXP 4, 63 rats were given ZnMet, ZnLys, or ZnSO₄ (30 mg Zn/kg) and CuLys, CuSO₄, or CuO (6 mg Cu/kg) in a 3 x 3 factorial EXP. After 4 wk, four rats from each T were sacrificed and the remaining rats fed a non-supplemented diet for 1 wk. Plasma Cu was lower for animals supplemented with CuO than CuSO₄ and CuLys. Bone Zn was higher for CuLys than CuO. The CuO T was lowest in BAV and kidney. Rats supplemented with CuSO₄ had higher muscle Cu than with CuLys. After depletion, plasma Cu was lower for CuO than CuLys, kidney Zn lower for CuSO₄ than for CuO which in turn had the lowest liver Cu. Amino acid complexed minerals were highly available. They were equal to and in some cases higher in BAV than the sulfate form and considerably more available in most cases than the oxide forms.

CHAPTER 1 INTRODUCTION

The benefits of mineral supplementation to animals have been known for a long time. In places where the fertility of the soils and nutritional quality of the feed is unknown or questionable, the use of mineral supplementation can provide security against deficiencies. The vast majority of the research to determine biological availability of minerals has been conducted with supplemental inorganic sources and with foodstuffs rather than with supplemental organic sources.

There are many methods to provide mineral supplementation which can be grouped into direct and indirect (McDowell, 1992). Indirect supplementation methods include the administration of minerals to the soils in the form of fertilizer or by changing the soil environment (e.g., pH, moisture, etc.) which may increase the availability of some minerals, or may encourage the growth of a specific pasture species which may contain more of the required minerals. Direct methods of supplementation include adding the supplemental minerals to the feed or water, or directly

injecting or placing the mineral inside the animal's body (McDowell, 1992).

Mineral supplements originate from different sources, with the most commonly used sources being inorganic in nature. It is known that different forms of inorganic minerals may be absorbed with varying efficiency.

Organic minerals are those that have an organic molecule (e.g., amino acid, carbohydrate, protein) attached to it. A chelated mineral is a compound which contains the metal bound to a synthetic molecule such as ethylenediaminetetraacetic acid (EDTA). These chelates are usually chemically stable and water soluble; however, the ligand is not biodegradable and, therefore, the mineral is not always bioavailable to the animal even after it is absorbed. In contrast, a metal-amino acid complex is the product resulting from complexing a soluble metal salt with an amino acid. The ligand formed in this complex is biodegradable and, therefore, the mineral may be more bioavailable.

Several products offering minerals in chelated form or amino acid complexes are available for mineral supplementation. Although considerable research has been done concerning the performance benefits, there have been fewer comparisons of minerals as amino acid complexes compared to their inorganic counterparts.

Some trials have determined ruminal degradation of different amino acid complexed minerals. There is, however,

a need to investigate the effect of these different sources on increasing concentrations of these minerals in the various fluids and tissues of the animal's body.

Zinc methionine, for example, has been determined to bypass ruminal degradation (Heinrichs and Conrad, 1983). Furthermore, since Zn is bound to methionine, it does not combine with any other substrate which may make it unavailable in the lumen of the animal and therefore it is ready for absorption as soon as it enters the small intestine. On the other hand, Spears (1989) observed that when Zn was deficient in the diet, apparent absorption of Zn from either Zn methionine or Zn oxide was similar. Zinc retention, however, was higher in the lambs fed the Zn methionine, this suggests a difference in the metabolism of these two sources following absorption. It has been hypothesized by Spears et al. (1991) that certain trace mineral chelates or complexes may enter different pools in the body than the inorganic forms.

In determining which parameters to evaluate for Zn and Cu metabolism research, it has been shown that the majority of biologically available Zn and Cu is stored in the organs of the body such as liver, kidney and pancreas with minor storage in the bone, muscle, skin and hair, although the latter two storage sites are not readily available to the animal. Blood plasma and blood cells serve as immediate sources of stored Zn and Cu. Furthermore, dietary Zn seems to affect the synthesis

of the metal inducible metalloprotein, metallothionein, in some tissues (Blalock et al., 1988).

The bioavailability of inorganic minerals has been measured under different situations for various species (Fox et al., 1981; Wedekind et al., 1992; Sandoval, 1992) by measuring several factors, such as tissue and fluid mineral concentration, and weight gain. There have been conflicting results concerning the bioavailability of the different Zn and Cu sources.

CHAPTER 2 LITERATURE REVIEW

Definitions of Trace Mineral Sources

There are many organic mineral sources. The natural organic mineral sources are those present in the environment. A mineral chelate is a metal complex in which the metal atom is held through more than one point of attachment to a ligand, with the metal atom occupying a central position in the metal complex (Morgan and Drew, 1920). Natural chelators are widely distributed in all living systems in nature. Some examples of mineral chelating agents include water, carbohydrates, proteins, amino acids, lipids, and nucleic acids.

Depending on the bond strength of the resulting compound or the ligand, it can then be classified as a chelate, complex, proteinate, or other moiety (Nelson, 1988). A list of these definitions is available (Kincaid, 1989). A proteinate is a product resulting from the chelation of a soluble salt with amino acids or partially hydrolyzed protein. A metal amino acid complex is a product resulting from the reaction of a metal ion from a soluble metal salt with a known amino acid.

The inorganic mineral sources are those which are not bound to organic molecules, but to other inorganic elements (e.g. sulfur, chloride, carbonate, oxide or the metal form itself).

Trace Mineral Bioavailability

Total concentration of a particular element in feed does not reflect the actual amount that will be absorbed by the animal. The reason is that frequently only a portion of that element will solubilize and then only a portion of that will be actually absorbed by the animal. Fox et al. (1981) defined bioavailability as a quantitative measure of the utilization of a nutrient under specified conditions necessary to support the organism's normal structure and physiological process. It is important to realize that just because an element is absorbed does not necessarily indicate that it will be utilized by the animal. The substance may not be metabolized for body function and may be excreted immediately (Bender, 1989). O'Dell (1985), therefore, offers a simplified definition of bioavailability which is the proportion of a nutrient in a feedstuff that can be absorbed and utilized.

Bioavailability of a compound implies the availability of that compound to some organism for body use (Miller, 1980). In trace elements, therefore, bioavailability refers to the portion which can be utilized by the animal to fulfill the functions for which the element is needed (Miller, 1980).

Factors that Affect Trace Mineral Bioavailability

It is impossible to understand mineral bioavailability without considering absorption. The events involved as a substance goes from its root uptake and later incorporation into the foodstuff to the actual fulfillment of a particular physiological function within the body of an animal are divided into three domains by Rosenberg and Solomons (1984): first, the luminal events which are responsible for the preparation and delivery of the substance for enterocyte uptake; second, the mucosal events which determine the transfer of the nutrient through the enterocyte to the basolateral membrane; and finally, the postabsorption events which include the transport, delivery, usage, and ultimately excretion of the nutrient.

The amount of a particular element available to the animal depends on both intrinsic and extrinsic factors (O'Dell, 1983) which have also been referred to as endogenous and exogenous (Rosenberg and Solomons, 1984). The intrinsic factors are physiological in nature and are much harder to control. They include species and genotype (Kincaid et al., 1976a, b), stage of production (Berg et al., 1963), age (Schisler and Kienholtz, 1967), physiological stress (Orr et al., 1990), nutritional status (Stuart et al., 1986), and intestinal well being (Bafundo et al., 1984). The extrinsic

factors are those which are present in the diet and include actual concentration of the diet, chemical or physical form of the element, presence of chelating agents, solubility of the source, presence of interacting nutrients, and protein concentration of the diet (Vohra and Kratzer, 1964; Rosenberg and Solomons, 1984; Stuart et al., 1986; Shafey et al., 1991). Overall, the extrinsic factors can be controlled more efficiently to improve bioavailability.

The many factors which have been studied for their possible effects on bioavailability include levels of supplementation of an element (Ammerman and Miller, 1972), elements supplied by different foods (O'Dell et al., 1972), different inorganic sources (Wedekind and Baker, 1990; Sandoval, 1992), and organic sources (Hill et al., 1986; Pimentel et al., 1991; Wedekind et al., 1992). Also, adsorption of minerals to macronutrients, binding of minerals to other compounds, and oxidation/reduction reactions may take place (van Dokkum, 1989). There are also individual genetic or physiological defects which can determine the absorption, or lack thereof, for any particular nutrient (Rosenberg and Solomons, 1984).

Assessment of Trace Element Bioavailability

O'Dell (1983) suggested that the best way to assess bioavailability was to compare absorption and utilization in a feedstuff with those in a standard soluble salt of the

element. Fox et al. (1981) suggested two indices of response, primary and secondary. Primary indices of response include quantifiable levels of morphology or physiological function that indicate health, like measures of growth (height, weight, head circumference, etc.), skeletal development (bone size, conformation, and mineralization), hematopoiesis, circulatory function, etc. Secondary indices of response are quantifiable responses that do not measure health status directly but must be correlated with primary indices under defined conditions, like whole body retention or concentrations of inorganic elements, metabolites, enzymes, or hormones in tissue, body fluids, or excretory products.

Comparisons of Organic and Inorganic Trace Element Sources

The use of inorganic mineral supplementation sources is well known throughout the industry. These products are used by many animal industries for their feed products as either direct mineral mixtures with the feedstuff or by manufacturing a separate mineral supplement. One of the most important factors determining the use of any particular source is its bioavailability within the target animal.

Zinc

The nutritional essentiality of Zn was demonstrated first in the rat (Todd et al., 1934). Nutritional interest in Zn was increased when the element was found to be deficient in

swine diets in 1955, poultry in 1958, cattle in 1960, and humans in 1961 (McDowell, 1992).

Zinc is a bluish white metal with atomic number 30 and an atomic weight of 65.37. Zinc is a divalent cation, with a specific gravity of 7.13 g/cm at 20°C, and melting and boiling points of 419.5 and 906°C, respectively. It is derived from numerous compounds, but the principal mineral ore is the sulfide sphalerite, which is the source of most metallic Zn (NRC, 1979).

The biochemical basis for the essentiality of Zn is not completely understood. Zinc metalloenzymes can be found in virtually every enzyme class (Vallee and Galdes, 1984). Several biological roles for Zn have been clarified, including those related to cell replication and differentiation (Hambridge et al., 1986). Zinc has also been postulated to have other roles independent of Zn metalloenzyme activity such as gene expression (Wu and Wu, 1987), membrane structure and function (Bettger and O'Dell, 1981), second messenger and protective agent in molecular storage systems (Grummt et al., 1986), and improvement of stability of human growth hormone (Cunningham et al., 1991). A great portion of the current research on Zn metabolism is aimed at its functions in molecular biology and nucleic acids.

Absorption

The specific gastrointestinal site where the majority of Zn is absorbed has not been identified (Solomons and Cousins, 1984). All of the sections of the small intestine may have a functional importance in Zn absorption (Cousins and Hempe, 1990).

Several investigators (Steel and Cousins, 1985; Hoadley et al., 1987) have reported that two kinetic processes are involved in absorption, passive diffusion and carrier-mediated components that may represent paracellular and intracellular absorption pathways. Carrier-mediated Zn absorption may increase during periods of low Zn intake, suggesting the stimulation of a carrier system to absorb greater amounts of Zn during a deficient state (Hoadley et al., 1987). In contrast, the diffusion component of Zn absorption is unaffected by Zn deficiency, and absorption via this process is proportional to luminal Zn concentration. Metallothionein (MT) synthesis is influenced both by dietary Zn level and by plasma Zn concentration and can regulate the quantity of Zn entering the body, thus playing a central role in Zn homeostasis (Cousins and Hempe, 1990).

Transport and Tissue Uptake

Albumin appears to be the main Zn carrier in blood with approximately 70% of the Zn bound to it (Vikbladh, 1950). Other Zn-containing components of plasma are α_2 -macroglobulin

(Parisi and Vallee, 1970), transferrin (Charlwood, 1979), and the amino acids cysteine and histidine (Morgan, 1981). Plasma Zn represents less than 1% of the total body content but serves as a primary source of the element accessible to all cells (Vallee and Falchuk, 1993). The exact mechanisms for tissue uptake of Zn are not well known. Tissue Zn concentration in most mammalian tissues has been reviewed (Hambridge et al., 1986), and tissue Zn levels were fairly constant among species. The tissues most sensitive to excess dietary Zn intake include liver, kidney, pancreas, small intestine, and bone (Kincaid et al., 1976a, b). Concentration changes of trace elements in tissues have been used as indicators of Zn bioavailability in rats and sheep (Moncilovic et al., 1975; Henry et al., 1988).

Bioavailability of Sources

The two predominant Zn sources used by the animal feed industry are ZnSO_4 (36% Zn) and ZnO (72% Zn). It has been suggested that the mineral source plays an important role in the formation of unknown complexes inside the digestive tract which in turn limit their absorption and further metabolism (Hughes, 1984; Clydesdale, 1990), but there are many contradicting studies as to the different effects and bioavailabilities of different sources. Zinc as the metal, sulfate, carbonate, oxide, and in several natural ores has been shown to be relatively available when provided in

suitable physical forms (Ammerman and Miller, 1972). In chicks, bioavailability of ZnO was determined to be 44.1% that of ZnSO₄ (Wedekind and Baker, 1990). In another study (Wedekind et al., 1992) Zn methionine (ZnMet) was reported to be better than both ZnSO₄ and ZnO.

In cattle, ZnMet is not broken down by ruminal microorganisms (Heinrichs and Conrad, 1983) and was found to be more bioavailable than ZnO (Chirase et al., 1991). In pigs, however, ZnMet was found to be of equal bioavailability with ZnSO₄ (Hill et al., 1986). In lambs, ZnO and ZnMet were absorbed to a similar extent, but were metabolized differently after absorption (Spears, 1989). In a summary of ZnMet studies by Herrick (1989), ZnMet increased gain and feed efficiency by an average of 3.5% in feedlot cattle. Also the addition of ZnMet to diets of lactating dairy cows has increased milk production and reduced somatic cell counts in milk (Herrick, 1989; Kellogg et al., 1989).

In determining performance and mineral metabolism of lambs, Kegley and Spears (1992) suggested ZnO and ZnSO₄ improved performance, but ZnMet had no effect. Chirase et al. (1992) determined that Zn and Mn methionine improve the recovery rates of calves stressed with infectious bovine rhinotracheitis virus. In lambs, the retention of a Zn proteinate source was higher than that of ZnO (Lardy et al., 1993). Visual hoof score and hoof durability have also been suggested to be affected by Zn supplement (Moore et al., 1992;

Reiling et al., 1992). Although hoof growth and wear measurements were similar for ZnMet supplemented dairy cows versus controls, visual hoof score (texture, heel cracks, laminitis, ulcers, interdigital dermatitis and hoof rot) showed improvement with ZnMet (Moore et al., 1992). Hoofs from Zn proteinate supplemented heifers had a higher shearing force than those from ZnSO₄ supplemented animals (Reiling et al., 1992). In young pigs, Hall et al. (1993) suggested increased availability of ZnMet versus ZnO supplementation. Rust and Schlegel (1993) reported no differences in steer performance or carcass characteristics with ZnO or ZnMet supplementation.

Copper

The nutritional essentiality of Cu was demonstrated first by McHargue (1925) based on the wide distribution of Cu in plant and animal tissues. Interest in Cu nutrition grew in the 1930s when Becker et al. (1931) and Neal et al. (1931) reported that Cu was responsible for a condition in Florida's cattle known as "salt sickness."

Copper has an atomic number 29 and an atomic weight of 63.55. Copper can exist as the metallic form or in +1, +2 or +3 valence states. The most common is the +2 state (Miller, 1979). It tends to occur in sulfide deposits, particularly igneous rocks, with concentrations in the continental crust of 50 ppm.

Understanding of the nutritional and metabolic roles of Cu is based on the functions of the known Cu-enzymes and on its role in disulfide bonding of keratin by an unknown mechanism; however, knowledge of its biological roles remains incomplete (Danks, 1988). In animals there are approximately ten proteins that are generally accepted as true cuproenzymes (Prohaska, 1988). In addition to the known cuproenzymes with specific functions, there are about 12 proteins of unknown functions that when isolated contain one or more Cu atoms (Prohaska, 1988). Some of the ten known cuproenzymes include: 1) tyrosinase (formation of melanin); 2) lysyl oxidase (synthesis of structural subunits of collagen and elastin); 3) dopamine β -hydroxylase (adrenal synthesis of catecholamine); 4) superoxide dismutase (immune, antioxidant function); 5) cytochrome C oxidase (energy metabolism via oxidative phosphorylation) (Allen and Solomons, 1984). Copper has also been suggested in the mineralization of growing bone, either in a cuproenzyme with ascorbate oxidase activity, or in its soluble ionic form (Hsieh and Hsu, 1980). A great deal of current research on Cu metabolism is focused on the use of Cu to improve immune response.

Absorption

In monogastrics, Cu is absorbed from all segments of the gastrointestinal tract including stomach and large intestine (Mason, 1979; Davis and Mertz, 1986). The major site of Cu

absorption is species dependant. The duodenum, however, has been generally accepted as the primary site for Cu absorption in most species (O'Dell, 1990).

The mechanism of Cu absorption is not clear, but absorption is known to be regulated at the intestinal mucosa. Passage of Cu across mucosal membrane and transport across cells are concentration-dependent and saturable and uptake by mucosal cells is not energy dependent (Crampton et al., 1965). Since MT has a stronger affinity for Cu than for Zn, the protein greatly influences Cu absorption in intestinal cells (Cousins, 1985). In adequate or high dietary Cu, when the animals demand of Cu is low, Cu enters the enterocyte and binds to MT preventing any additional uptake. With low dietary Cu, the MT bound Cu present in the intestine would have been released through the basolateral membrane to the portal vein and consequently taken to the liver. Sheep, however, are more sensitive to Cu toxicosis because of the lack of intestinal MT synthesis (Saylor et al., 1980). Furthermore, Turner et al. (1987) working with everted sacks of sheep jejunum, suggested that Cu uptake from lumen to cells was a process neither saturable nor energy-dependant but whose kinetics reflected that of simple diffusion. Another important factor in Cu homeostasis in sheep is that sheep cannot excrete high amounts of Cu in bile acids (Gooneratne et al., 1989).

One of the most powerful antagonists of Cu absorption in general seems to be Zn (O'Dell, 1985). Excess dietary Zn has been reported to aggravate the signs of low Cu status (L'Abbe and Fischer, 1984). In rats fed adequate Cu levels (6 mg/kg), Zn dietary concentrations of 120 and 240 mg/kg depressed the activities of important cuproenzymes such as liver superoxide dismutase and heart cytochrome C oxidase (L'Abbe and Fischer, 1984). This antagonistic effect appears to take place mainly in the intestinal mucosa via MT (Cousins, 1985).

Transport and Tissue Uptake

As with Zn, albumin appears to be the main Cu carrier in portal blood. After passing through enterocytes, Cu is transported through portal blood as a histidine-Cu-albumin complex (Lau and Sarkar, 1971). There are controversial reports as to the actual carrier of Cu in peripheral circulation. After transport into hepatocytes, Cu is released into the blood stream bound mostly to ceruloplasmin. Copper seems to stay bound to ceruloplasmin through peripheral circulation (O'Dell, 1990). Bremner (1980), however, suggests that the principal transport forms of Cu are its loosely bound complexes with albumin and, to a lesser extent, to selected amino acids which include histidine, threonine and glutamine. Hepatic Cu is temporarily stored complexed to ceruloplasmin and released into plasma or bile as such (Bremner, 1980).

Other suggested storage proteins in liver include superoxide dismutase, MT, and mitochondriocuprein (Bremner, 1980).

Bioavailability of Sources

Supplementation of Cu as CuSO_4 , CuCO_3 , CuCl_2 or $\text{Cu}(\text{NO}_3)_2$ resulted in similar elevations in blood and plasma Cu concentrations in sheep (Lassiter and Bell, 1960) and cattle (Chapman and Bell, 1963), but both Cu_2O and CuO forms were less available (Lassiter and Bell, 1960). In the growing chick, Cu from CuI and Cu_2O were 82 and 76%, respectively, as available as CuSO_4 (McNaughton et al., 1974). According to Ho et al. (1980), amino acid complexes or organically bound forms apparently have a higher bioavailability than inorganic Cu sources because of their ability to prevent the occurrence of hypocupremia in beef cattle.

Other recent studies with swine (Cromwell et al., 1989), chicks (Baker et al., 1991), sheep (Pott et al., 1992) and cattle (Clark et al., 1993) show that CuSO_4 is more available than CuO . Baker et al. (1991), however, suggested similar bioavailability for Cu from a Cu lysine (CuLys) complex to that of CuSO_4 in chicks. Liver Cu has been reported to increase more rapidly when cattle were supplemented a Cu proteinate versus CuSO_4 (Clark et al., 1993). In studies with weanling pigs (Coffey et al., 1992; Coffey et al., 1993), it was suggested that CuLys and CuSO_4 were not only similar for many aspects of bioavailability but that CuLys was also an

effective growth promotant for weanling pigs. In sheep studies (Pott et al., 1992), Cu from CuCl_2 , CuSO_4 , CuCO_3 , and Cu acetate sources were of similar bioavailability. Nockels et al. (1993) suggest that CuLys is better retained than that of CuSO_4 in stressed calves and that significant changes occurred in Cu and Zn balance with supplementation and stress.

In a bioavailability study using growing cattle, Kegley and Spears (1993) suggested that Cu from CuLys was of similar bioavailability to that from CuSO_4 but these were higher than CuO. Kincaid et al. (1986) using a diet high in Mo and S found Cu proteinate to increase Cu status (plasma and liver Cu) more readily than CuSO_4 suggesting the Cu proteinate to be less affected by high Mo. In two separate studies, however, Wittenberg et al. (1990) showed no difference between Cu proteinate and CuSO_4 as to their effect on the Cu status of Cu depleted steers fed diets high in Mo. Ward et al. (1993) supplemented CuLys or CuSO_4 to growing steers fed a diet with or without supplemental Mo and S. They found no difference between CuLys and CuSO_4 bioavailability using growth rate, feed intake, feed efficiency, plasma Cu, ceruloplasmin activity, and immune response as indicators of Cu status.

Bioavailability of Other Trace Mineral Complexes

Ward et al. (1992) suggested that a mixture of Zn, Mn, Cu and Co in amino acid complexed forms may stimulate feed intake and growth during the initial stress period of feedlot steers

compared to the oxide or sulfate forms. A 75% inorganic and 25% proteinate Zn, Mn and Cu mixture provided as a dietary supplement improved embryo and/or fetal survival, and reduced the duration of estrus in sows compared to those supplemented with an organic mixture (Mirando et al., 1993).

Spears (1991) showed, feeding a Mn deficient diet to heifers, how Mn methionine (MnMet) supplementation was superior to MnO in improving growth and feed efficiency. Henry et al. (1992) suggested MnMet to be equally available to MnSO₄ but more available than MnO. The relative bioavailability of Mn proteinate was similar to that of MnSO₄ in chicks fed diets either devoid of or containing fiber and phytate (Baker and Halpin, 1987). Also in chicks, MnMet was 174% more available (based on bone Mn accumulation) than MnO (Fly et al., 1989), and in another study (Henry et al., 1989) MnMet was not only more available than MnO but also than MnSO₄.

Organic iodine (ethylenediamine dihydroiodide) supplemented mice had similar macrophage phagocytosis to NaIO₃ and NaI supplemented mice (Siddiqui et al., 1993).

In a toxicity and tissue retention study conducted with rats, Na₂SeO₄ was found to be more toxic to methionine deficient rats than L-selenomethionine (SeMet) (Salbe and Levander, 1990). For pigs, however, SeMet was more toxic than the inorganic form (Herigstad et al., 1973). Furthermore, SeMet has been shown to protect chicks against pancreatic

atrophy more effectively than inorganic Se (Cantor et al., 1975).

In supplementing a complexed form of Co (Co dextro lac) to feedlot cattle, Carpenter et al. (1992) suggested no benefits in terms of animal performance versus no supplementation. Using growing-finishing pigs, Mooney and Cromwell (1993) indicated that Cr picolinate resulted in carcasses with increased percentages of muscle and decreased percentages of fat versus controls.

CHAPTER 3
RELATIVE BIOAVAILABILITY OF ZINC METHIONINE
AND TWO INORGANIC ZINC SOURCES
FED TO CATTLE

Introduction

Several products offering minerals in chelated form or complexed with amino acids are available for mineral supplementation. Zinc methionine (ZnMet) can bypass ruminal degradation (Heinrichs and Conrad, 1983). Furthermore, it does not combine with any other substrate which may render it unavailable in the lumen of the animal and, therefore, it is ready for absorption upon entering the small intestine. Spears (1989) found that when a deficient diet was fed, apparent absorption of Zn from ZnMet or ZnO was similar, but Zn retention increased by ZnMet suggesting different metabolism following absorption. Spears et al. (1991) hypothesized that organic sources enter different body pools than inorganic forms. The popular Zn sources used by the animal feed industry are ZnSO_4 (36% Zn) and ZnO (72% Zn); therefore, it is necessary to test other products against those currently being used.

The majority of bioavailable Zn when supplemented in relatively high levels is stored in body organs such as liver,

kidney and pancreas with minor storage in bone, muscle, skin and hair (Ott et al., 1966). Blood plasma and blood cells serve as immediate sources of stored Zn. Increasing dietary Zn has also been shown to stimulate the production of the protein metallothionein (MT) in some tissues (Blalock et al., 1988).

The present study was undertaken to compare the effect of supplemental ZnMet, ZnSO_4 , and ZnO on Zn, Cu and MT concentrations in various fluids and tissues of the animal's body.

Materials and Methods

Thirty-two yearling Limousine and Angus cross-bred heifers ranging from 213 to 318 kg and averaging 256 ± 31 (mean \pm standard error of the mean; SEM) kg were used in a 12 wk experiment. Four wks prior to the experimental period animals were randomly assigned and housed in 4 earth pens (511 m^2 , eight animals per pen) for 2 wk. This provided an adjustment and training period in which heifers received a diet without Zn supplementation and low quality Bermuda grass hay in order to minimize Zn stores. Subsequently, treatments were randomly assigned to animals. The treatments consisted of three different Zn sources to supply 360 mg/d of supplemental Zn: ZnMet (Zinpro Corporation, Edina, MN), ZnSO_4 or ZnO (Southeastern Minerals, Bainbridge, GA) and a negative control group which received no supplemental Zn. To provide

the daily supplemental Zn the diets were formulated to contain 200 mg Zn/kg diet. Animals were fed individually via Calan gates 1.8 kg of a corn-based concentrate into which the three different Zn sources were mixed. Bermuda grass hay was offered ad libitum. Zinc content (Dry matter basis, DMB) was 19.6 mg/kg in hay and ranged from 20 to 26 mg/kg in the control diet (Table 3-1). The diet was formulated to be adequate in protein, energy, vitamins, and minerals for this class of cattle (NRC, 1984). Heifers were given supplemental Zn for 4 wks, depleted (not supplemented) the following 4 wks and then supplemented for 4 wks. Protocol for animal care had been approved by the University of Florida's Institutional Animal Care and Use Committee. Weights were obtained monthly

TABLE 3-1. Composition of concentrate diet offered to cattle (as fed)^a.

Ingredient	Percentage
Corn	84.07
Soybean meal (44% CP)	14.30
Dicalcium phosphate	1.05
Salt ^b	.29
Trace mineral mix ^c	.26
Vitamins A and D ₃ ^d	.03

^a Zn analysis biweekly indicated means of 248, 251 and 256 mg/kg for ZnMet, ZnSO₄ and ZnO, respectively. Hay intake ranged from 2 to 5 kg (as-fed).

^b Provided 2.1 g of NaCl per kilogram of diet.

^c Provided .24 mg of I (KI), .28 mg of Co (CoCO₃), 82 mg of Fe (FeSO₄), 38 mg of Cu (CuCl), 48 mg of Mn (MnSO₄) and **no** Zn per kg of diet.

^d Provided 5,000 IU of vitamin A and 500 IU of vitamin D₃ per kg of diet.

and blood samples were obtained via jugular venipuncture on d 1 before animals were administered the concentrate and thereafter on d 3, 14 and biweekly. To obtain serum, samples were centrifuged at 700 x g for 25 min, supernatant decanted and frozen until analyzed for Zn and Cu levels. Erythrocytes were harvested from centrifuged (700 x g for 25 min) whole blood from which plasma had been removed and had been washed twice with 9 M cold saline solution. An erythrocyte lyase was prepared by combining 1 ml erythrocytes with 1.5 ml deionized water and frozen for storage. At the end of the experiment on d 84, animals were stunned with a captive bolt shot and euthanized by exsanguination.

Liver, pancreas, kidney, bone and bone marrow (metacarpus), skin, hair, hoof, neck muscle (sterno mandibularis), and eye were excised and frozen for further mineral analyses. Zinc and Cu in tissues, blood constituents and diet samples collected were determined by air acetylene flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with AS-50.

Metallothionein was measured in liver, pancreas and kidney by the $^{109}\text{Cd}^{2+}$ -hemoglobin affinity assay (Eaton and Toal, 1982). For this procedure .2 g of the tissue was homogenized with 4 volumes of cold 10mM Tris-HCl buffer (pH 7.4), with a Potter-Elvehjem glass-teflon tissue grinder, and centrifuged (40,000 x g; 10 min; 4°C). The supernatant was then heated (100°C; 5 min) and centrifuged (10,000 x g; 5 min). Next, 200

μl of Cd solution (2 μg Cd and .5 μCi ^{109}Cd per ml of 10 mM Tris-HCl buffer, pH 7.4) were added to a 200 μl aliquot of the supernatant and the mixture was incubated (10 min; room temperature). Finally, 100 μl of 2% hemoglobin were added. The sample was then heated (100°C; 2 min) and centrifuged (10,000 \times g; 5 min). This step was then repeated and 100 μl of the supernatant and standard solutions were placed in a Gamma Spectrometer Model 4000 (Beckman Instruments, Inc.) to measure ^{109}Cd levels. These levels were then used to assess MT concentrations.

The experiment was designed as a 4 \times 4 factorial in a completely randomized experiment. There were four Zn treatments, three sources of Zn and one negative control, and four levels of vitamin E. All data were analyzed using SAS (SAS, 1988). Repeated measures ANOVA was performed using the general linear model (GLM) procedure on changes (increase or decrease from d 1) in Zn and Cu concentrations in serum, and on changes in Zn concentrations in erythrocytes. Tissue Zn and Cu data were analyzed using GLM. In case of significance ($P < .05$) in serum or tissue data, Waller-Duncan's K-ratio T test was used for multiple comparisons.

Results

Since no zinc \times vitamin E interaction was found the statistical evaluation was based on main effects. The vitamin E data were discussed by Njeru et al. (1993). Weight gains

were not different ($P > .05$) among treatments. Concentrate intakes were similar for all groups with no anorexia noted.

There were no treatment differences ($P > .05$) in serum Zn content for all days of collection (Figure 3-1), with the amount of dietary Zn not playing any role in controlling serum Zn, since the unsupplemented controls were not different. Surprisingly, on d 28 (beginning of depletion phase) serum Zn in most treatments (including the control) started increasing and upon repletion (d 56) levels started falling again. As with serum Zn, erythrocyte Zn was not affected ($P > .05$) by treatment (Figure 3-2). Unlike serum Zn, however, Zn content of erythrocytes dropped in all treatments with depletion and stabilized with repletion.

Overall mean serum Cu concentrations fluctuated greatly but tended to decrease with all Zn treatments (Figure 3-3). By d 56 the Cu concentration increased (from d 1) by $.17 \pm .07$ $\mu\text{g/ml}$ (mean \pm SEM) for ZnO treatment and was greater ($P < .05$) than both ZnMet and control treatments which dropped by $-.04 \pm .05$ and $-.05 \pm .07$ $\mu\text{g/ml}$, respectively. By d 70 the Cu concentrations for the ZnO treated sheep ($-.02 \pm .13$) had decreased less ($P < .05$) than other treatments.

There were no treatment differences ($P > .05$) in Zn (Table 3-2) and Cu (Table 3-3) tissue concentrations and liver, kidney and pancreas MT concentrations (Table 3-4). As with the blood data, no differences were seen among Zn sources or even between the supplemented treatments and the

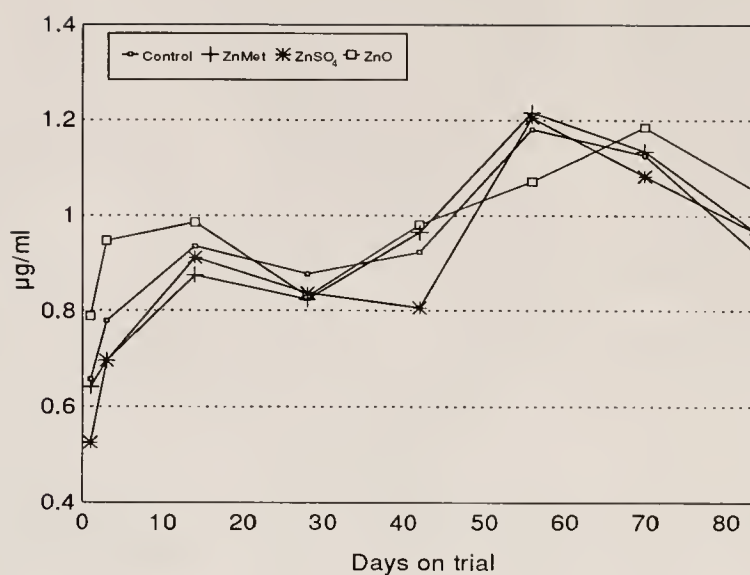


FIGURE 3-1. Mean serum Zn for cattle supplemented with different sources of Zn. SEM ($\mu\text{g/ml}$) for d 1 .20, d 3 .18, d 14 .25, d 28 .21, d 42 .21, d 56 .36, d 70 .32, d 84 .29.

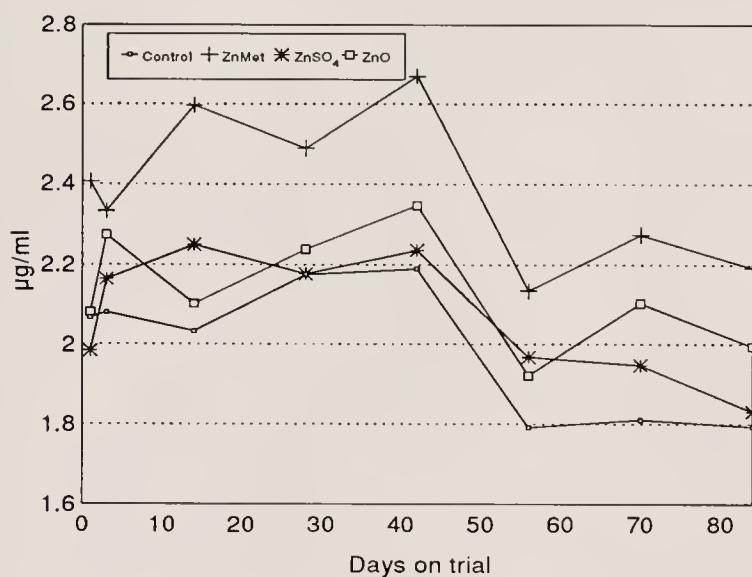


FIGURE 3-2. Mean erythrocyte Zn for cattle supplemented with different sources of Zn. SEM ($\mu\text{g/ml}$) for d 1 .45, d 3 .43, d 14 .31, d 28 .33, d 42 .34, d 56 .33, d 70 .40, d 84 .36.

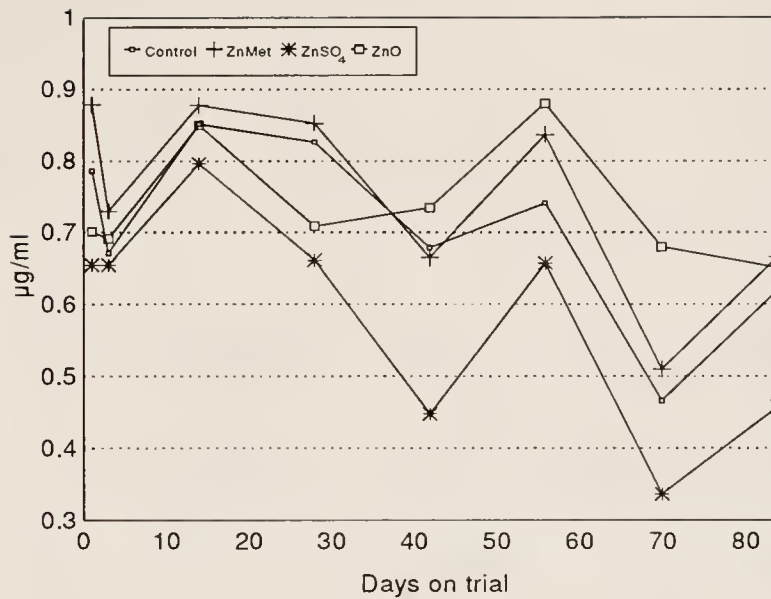


FIGURE 3-3. Mean serum Cu for cattle supplemented with different sources of Zn. SEM (µg/ml) for d 1 .20, d 3 .15, d 14 .18, d 28 .21, d 42 .24, d 56 .16, d 70 .24, d 84 .16.

TABLE 3-2. Mean Zn concentrations in tissues of cattle supplemented with three sources of Zn (mg/kg, DMB^a)

Tissue ^b	Control	ZnMet	ZnSO ₄	ZnO	SEM
Bone ^d	60	60	61	62	8
Bone Marrow ^c	143	137	133	133	22
Cornea	2.8	2.3	3.2	2.8	.97
Skin	1.9	1.5	1.8	2.2	1.4
Hair	86	88	86	84	8
Hoof	77	74	70	81	10
Kidney	72	67	69	69	5
Liver	111	116	118	114	18
Muscle ^d	190	179	182	192	25
Pancreas	65	66	66	62	10

^a DMB= Dry matter basis, bone also fat free, cornea and skin on wet basis.

^b No difference among treatments (P > .05)

^c Metacarpus.

^d Sterno mandibularis.

TABLE 3-3. Mean Cu levels in tissues of cattle supplemented with three sources of Zn (mg/kg, DMB^a)

Tissue ^b	Control	ZnMet	ZnSO ₄	ZnO	SEM
Bone	6	6	5	6	1.4
Bone Marrow	28	30	28	30	7
Hair	6	6	6	6	.9
Hoof	2.2	1.9	2.0	2.2	.8
Kidney	17	17	16	16	3
Liver	172	163	215	150	56
Muscle	4	4	4	4	1
Pancreas	4	4	5	5	1

^a DMB= Dry matter basis, bone also fat free basis.

^b No difference among treatments (P > .05).

TABLE 3-4. Mean metallothionein levels in tissues of cattle supplemented with three sources of Zn (μg MT/g^a)

Tissue ^b	Control	ZnMet	ZnSO ₄	ZnO	SEM
Liver	140	125	108	139	57
Kidney	68	63	63	56	19
Pancreas	43	46	29	44	18

^a μg MT/g= μg of Metallothionein per gram of wet tissue.

^b No difference among treatments (P > .05)

unsupplemented control. Copper concentrations of the tissues did not drop in the supplemented treatments as might have been expected when compared to controls and stayed similar throughout treatments and within tissues.

Discussion

Since dietary Zn levels were relatively high this may have accounted for lack of difference among Zn sources. Animals were receiving up to 80 mg of Zn/d from the control diet alone. On the other hand both the hay (19.6 mg of Zn/kg) and the concentrate (20 to 26 mg of Zn/kg) were below the minimum adequate level of 30 mg/kg (NRC, 1984). Spears (1989) showed increased retention of Zn in lambs supplemented with ZnMet compared to ZnO. In this study no differences were observed in serum Zn concentrations even when all the animals were given the same amount of Zn for the 4 wks of depletion. It would have been beneficial, however, to decrease the Zn level in the basal diet to try to stimulate Zn mobilizing mechanisms. There were a few unexplainable serum Cu differences on d 56 and 70. These could be related to stress since ceruloplasmin induction produced by stress would elevate serum Cu levels (Cousins, 1985).

Availability of Zn sources are in agreement with other researchers in studies with swine (Hill et al., 1986) and chicks (Pimentel et. al., 1991). These researchers indicated no differences in availability between organic and inorganic sources of Zn. Wedekind and Baker (1990) showed increased bone Zn deposition in chicks fed ZnMet relative to ZnO and ZnSO₄. Wedekind et al. (1992) also suggested that bone Zn levels increased when ZnMet was used compared to ZnSO₄ or ZnO, however, they did not use fat-free bone in their study. Lack

of a difference among sources in the present study is also in agreement with a sheep experiment to determine if Zn from ZnMet would influence muscle (longissimus or biceps femoris) Zn concentrations (Medeiros et al., 1989). A genetic difference might also exist between the species used by other researchers in terms of their Zn metabolism especially between ruminants and monogastrics. It is suggested that this trial be conducted using similar levels of supplemental Zn in combination with lower levels of basal dietary Zn. This kind of study would be more expensive because it would necessitate the use of purified or semipurified diet.

Implications

These results suggest that at adequate levels of dietary Zn, bioavailability of supplemental Zn sources may be less important than under conditions of limited dietary Zn or increased supplemental Zn.

Summary and Conclusions

A 12 wk experiment was conducted to compare supplemental ZnMet, ZnSO₄, and ZnO on Zn, Cu and MT concentrations in various fluids and tissues of 32 yearling cattle. Supplemental Zn (360 mg/d) was fed for 4 wks, withdrawn for 4 wks and then resumed for another 4 wks. Mineral (Zn and Cu) concentrations were determined in serum, liver, pancreas, kidney, bone, bone marrow, hair, hoof and neck muscle, and Zn

only in erythrocytes, skin, and cornea. Metallothionein levels were determined in liver, pancreas and kidney. There were no treatment differences ($P > .05$) in serum or erythrocyte Zn content for all days of collection. Serum Cu concentrations tended to decrease with all treatments. There were no treatment differences ($P > .05$) in Zn and Cu tissue concentrations and liver, kidney and pancreas MT concentrations. Tissue Cu concentrations did not drop in the supplemented treatments when compared to controls. At adequate levels of dietary Zn, bioavailability of supplemental Zn sources may be less important than under conditions of limited dietary Zn or if very high levels of supplemental Zn are fed.

CHAPTER 4
RELATIVE BIOAVAILABILITY OF TWO ORGANIC
AND TWO INORGANIC ZINC SOURCES
FED TO SHEEP

Introduction

The use of amino acid complex minerals in mineral supplements compared to inorganic forms is still controversial. Limited research has been done concerning the biological availability of organic and inorganic mineral sources. In a trial to test bioavailability, Spears (1989) found that when a deficient diet was fed, apparent absorption of Zn from Zn methionine (ZnMet) or ZnO forms was similar, but Zn retention increased with ZnMet, suggesting different metabolism following absorption.

The majority of bioavailable Zn when supplemented in relatively high levels is stored in body organs such as liver, kidney and pancreas with minor storage in bone, muscle and skin (Ott et al., 1966). Blood plasma serves as an immediate source of stored Zn. Dietary Zn also stimulates production of the protein metallothionein (MT) in some tissues (Blalock et al., 1988).

The objectives of this study were to compare bioavailability of two organic and two inorganic Zn sources in

sheep by evaluating Zn and Cu concentrations of selected tissues and serum, and MT in kidney, pancreas and liver.

Materials and Methods

Forty crossbred wether lambs averaging 37.6 ± 3.1 kg (mean \pm SEM) were randomly assigned to one of five treatments. The treatments consisted of four different sources to supply 360 mg/d of supplemental Zn: ZnMet, Zn lysine (ZnLys; Zinpro Corporation, Edina, MN), ZnSO₄ or ZnO (Southeastern Minerals, Bainbridge, GA) and a negative control group which received no supplemental Zn. The basal diet contained from 16 to 20 mg/kg Zn (Table 4-1). The diet was formulated to be adequate in protein, energy, vitamins, and minerals for this class of sheep (NRC, 1985).

Lambs were housed in individual wooden pens (1.4 m²) with expanded metal floors in an open sided barn. Feed intake was restricted to 1000 g/hd daily (as-fed basis) with tap water available ad libitum. The protocol for animal care had been approved by the University of Florida's Institutional Animal Care and Use Committee.

Lambs were fed the treatment diets for 3 wks following a 7 d adjustment period during which all the animals were fed the basal diet. After the first supplementation period, animals were not supplemented with any additional Zn for 4 wks and then supplementation was resumed for the last wk.

Animals weights were obtained at the beginning and at the end of the experiment. Blood samples were obtained via jugular venipuncture on d 0 before diet administration and on d 14, 21, 28, 49 and 55. To obtain serum, samples were centrifuged at 700 x g for 25 min, supernatant decanted and frozen until analyzed for Zn and Cu levels. At the end of the experiment on d 55, animals were stunned with a captive bolt shot and euthanized by exsanguination.

Liver, pancreas, kidney, bone and bone marrow (metacarpus), skin, hoof, leg muscle (flexor carpi ulnaris), and eye were excised and frozen for further mineral analyses. Zinc and Cu in tissues, blood constituents and diet were

TABLE 4-1. Composition of basal diet offered to sheep (as fed)^a

Ingredient	Percentage
Ground yellow corn	59
Cotton seed hulls	21
Soybean meal (44% CP)	12
Alfalfa meal (14% CP)	3
Corn oil	3
Trace mineral salt ^b	1
Ground limestone	1
Vitamins A and D ₃ ^c	.0008

^a Zn analysis biweekly indicated means of 415, 444, 446 and 421 mg/kg for ZnO, ZnSO₄, ZnLys and ZnMet, respectively.

^b Provided .24 mg of I (KI), .28 mg of Co (CoCO₃), 82 mg of Fe (FeSO₄), 38 mg of Cu (CuCl), 48 mg of Mn (MnSO₄) and **no** Zn per kg of diet.

^c Provided 5,000 IU of vitamin A and 500 IU of vitamin D₃ per kg of diet.

determined by air acetylene flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with AS-50. Metallothionein was measured in liver, pancreas and kidney by procedures previously described (chapter 3).

All data were analyzed using SAS (SAS, 1988). Repeated measures ANOVA was performed using the general linear model (GLM) procedure on changes (increase or decrease from d 1) in serum Zn and Cu concentrations. Tissue Zn and Cu data were analyzed using GLM procedure. In case of significance ($P < .05$) of either serum or tissue mineral concentrations, Waller-Duncan's K-ratio T test was used for multiple comparisons (Waller and Duncan, 1969).

Results

Three of the lambs (2 from the control group and 1 from the sulfate group) died from unrelated causes. Weight gains were not different ($P > .05$) among treatments. Feed intakes were similar for all groups with no anorexia noted.

When compared to d 1, d 49 serum Zn concentrations had increased by $.20 \pm .13 \mu\text{g/ml}$ (mean \pm SEM) for control sheep which was lower ($P < .05$) than those of ZnLys, ZnSO_4 , and ZnO treatments which had increased by $.74 \pm .12$, $.62 \pm .07$, and $.83 \pm .12 \mu\text{g/ml}$, respectively, but was not lower than ZnMet which increased by $.52 \pm .16 \mu\text{g/ml}$ (means shown on Figure 4-1). Treatment differences were also seen on d 55 with a higher ($P < .05$) serum Zn increase for ZnLys ($1.58 \pm .28$

µg/ml) than ZnMet, ZnO or control treatments ($.78 \pm .27$, $.62 \pm .1$, and $.75 \pm .26$ µg/ml, respectively), but not ZnSO₄ ($.87 \pm .17$ µg/ml). During the depletion phase (d 21 to 49) mean serum Zn levels did not differ ($P > .05$) from those levels before the beginning of depletion (d 21). Overall serum Cu levels fell slightly with all treatments (Figure 4-2). Most serum concentrations were, however, above the minimum critical level of .65 µg/ml, suggested by McDowell et al. (1984). There were no treatment effects ($P > .05$) for any of the sampling days.

The ZnLys treatment had the highest ($P < .05$) Zn accumulation (581, 389, and 340 mg/kg) for kidney, liver and pancreas, respectively (Table 4-2). Both ZnSO₄ and ZnMet treatments had higher ($P < .05$) liver Zn concentrations (195 and 198 mg/kg, respectively) than the control treatment (127 mg/kg). Liver Zn concentrations for ZnO were not different ($P > .05$) than control, ZnSO₄ or ZnMet. Kidney Zn concentrations of both ZnSO₄ and ZnMet treatments tended ($P < .15$) to be higher than controls. The remaining Zn levels for bone, bone marrow, cornea, skin, hoof and muscle were not different ($P > .05$) among treatments. Most of the Zn concentrations for those tissues were relatively constant among treatments.

Response to treatments in terms of tissue MT (Table 4-3) were very similar to that of tissue Zn levels. The ZnLys treatment had the highest ($P < .05$) MT levels of 79, 167, and 68 µg MT/g for liver, kidney, and pancreas, respectively.

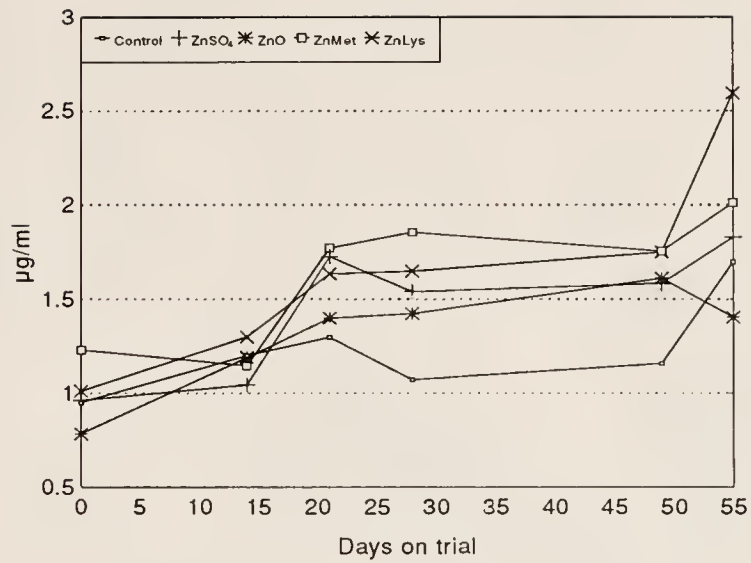


FIGURE 4-1. Mean serum Zn levels for sheep supplemented with different sources of Zn. SEM ($\mu\text{g/ml}$) for d 0 .40, d 14 .40, d 21 .43, d 28 .59, d 49 .34, d 55 .63.

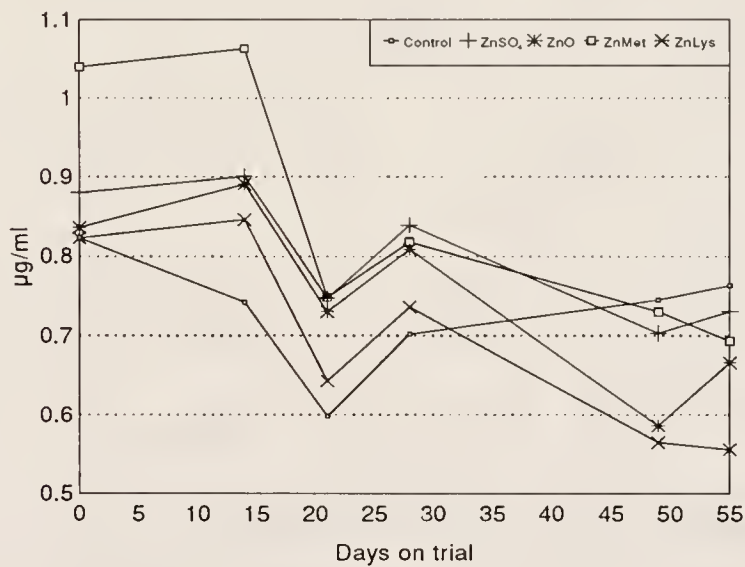


FIGURE 4-2. Mean serum Cu levels for sheep supplemented with different sources of Zn. SEM ($\mu\text{g/ml}$) for d 0 .15, d 14 .16, d 21 .18, d 28 .15, d 49 .20, d 55 .23.

TABLE 4-2. Mean Zn concentrations in tissues of sheep supplemented with four sources of Zn (mg/kg, DMB^a)

Tissue	Control ^b	ZnO ^c	ZnSO ₄ ^d	ZnMet ^c	ZnLys ^c	SEM ^e
Bone	83	98	97	96	91	13
Bone Marrow	63	79	79	84	73	21
Cornea	7	6	6	7	8	2
Skin	24	27	26	26	31	9
Hoof	94	112	113	105	89	59
Kidney	117 ^f	137 ^f	234 ^f	226 ^f	581 ^g	131
Liver	127 ^f	140 ^{f,g}	195 ^g	198 ^g	389 ^h	66
Muscle	260	257	261	259	267	22
Pancreas	86 ^f	107 ^f	139 ^f	135 ^f	340 ^g	110

^a DMB= Dry matter basis, bone also fat free, cornea and skin on wet basis.

^b n=6. ^c n=8. ^d n=7.

^e SEM = Standard Error of the Mean.

^{f,g,h} Means with different subscripts across row differ (P < .05).

TABLE 4-3. Mean metallothionein content of tissues of sheep supplemented with four sources of Zn (µg MT/g^a)

Tissue	Control ^b	ZnO ^c	ZnSO ₄ ^d	ZnMet ^c	ZnLys ^c	SEM ^e
Liver	2.0 ^f	4.5 ^f	11 ^f	8.2 ^f	79 ^g	21
Kidney	4.8 ^f	14 ^f	45 ^f	45 ^f	167 ^g	50
Pancreas	1.7 ^f	2.6 ^f	7.9 ^f	11 ^f	68 ^g	36

^a µg MT/g= µg of Metallothionein per gram of wet tissue.

^b n=6. ^c n=8. ^d n=7.

^e SEM = Standard Error of the Mean.

^{f,g} Means with different subscripts across row differ (P < .05).

Likewise, both ZnSO₄ and ZnMet treatments resulted in higher concentrations than both the control and ZnO groups in the three tissues but differences were not significant (P > .05).

TABLE 4-4. Mean Cu levels in tissues of sheep supplemented with four sources of Zn (mg/kg, DMB^a)

Tissue	Control ^b	ZnO ^c	ZnSO ₄ ^d	ZnMet ^c	ZnLys ^c	SEM ^b
Bone	1.0	.9	.9	1.2	1.1	.3
Bone Marrow	9	10	11	10	6	7
Hoof	3.2	3.3	3.5	3.5	2.4	1.9
Kidney	41	41	66	43	53	31
Liver	1076	1299	1365	1239	1201	381
Muscle	10 ^f	7 ^g	6 ^g	5 ^g	6 ^g	2.3
Pancreas	13	9	10	5	9	4

^a DMB= Dry matter basis, bone also fat free basis.

^b n=6. ^c n=8. ^d n=7.

^e SEM = Standard Error of the Mean.

^{f,g} Means with different subscripts across row differ (P < .05).

Most tissue Cu concentrations did not differ (P > .05) among treatments and remained relatively constant. Mean muscle Cu concentration (10 mg/kg), however, was highest (P < .05) for the control group. There was a large Cu accumulation in the livers of these animals and was probably due to the high dietary Cu (70 mg/kg) levels.

Discussion

Unlike in chapter 3, where Zn intake was a minimum of 75 mg/d, Zn levels for the basal diet (16 to 20 mg/kg) for this trial with sheep were well below the marginal level of 30 mg/kg (NRC, 1985). Furthermore, since the animals were only given 1 kg of feed, their actual Zn intake from the basal diet was only 16 to 20 mg/d.

Serum Zn concentrations increased with supplementation, but these levels did not decrease during the month of depletion. Therefore, it is impossible to speculate from these data about tissue Zn retention by the different treatment groups. Increased retention of Zn in lambs fed ZnMet had been shown to be greater than that from ZnO treatment (Spears, 1989). Mean serum Zn levels were different on d 49 and 55. Furthermore, on d 55 Zn levels for the ZnLys treatment had increased more from d 1 than those of all treatments except ZnSO₄. This may imply a higher bioavailability for these two sources.

Serum Cu concentrations dropped slightly during the trial. This was unexpected since the basal diet contained around 70 mg/kg Cu to prevent the adverse effects of the high levels of Zn supplementation. Since serum Cu content was not lowered by the high Zn content of the Zn supplemented treatments, this suggests different absorption routes for these elements. Furthermore, decreased serum Cu levels were unexpected because of the accumulation of Cu in the liver of all the animals. Therefore, the low serum Cu may have resulted from low hepatic Cu mobilization. There were, however, no signs of Cu toxicity in any of the animals studied.

The data suggest that ZnLys has greater bioavailability as a source of supplemental Zn. This suggestion is made on the basis of greater accumulation of Zn in the liver, kidney

and pancreas of the ZnLys treated animals. The other organic source (ZnMet) was not different than ZnSO₄. There is a possibility that if the ZnLys treatment had been omitted there would be differences between the ZnMet and ZnSO₄ groups compared to the ZnO and control groups, due to the high values of the ZnLys group particularly for liver and kidney Zn and MT levels, which might imply different variances for the different means. No differences were observed in the bone Zn deposition for the various treatments. This is unlike the work of Wedekind and Baker (1990) which showed increased bone Zn deposition in chicks fed ZnMet relative to ZnO and ZnSO₄. Wedekind et al. (1992) also suggested that bone Zn levels increased when ZnMet was used compared to ZnSO₄ or ZnO in chicks, however, they did not use fat-free bone in their study. Results reported herein (no differences) also agree with those of Medeiros et al. (1989) who found that ZnMet did not influence muscle (longissimus or biceps femoris) Zn content.

Metallothionein determination was valuable in assessing the differences among Zn sources. Mean liver, kidney and pancreas MT levels from the ZnLys treatment ranged anywhere from about 3 to 40 times greater than the other treatments. The closest values were those for ZnMet and ZnSO₄ and the lowest values were usually those of the negative control group which was expected. These MT determinations confirm the poor biological value of ZnO, with tissue concentrations associated

with this treatment being very close to the control treatment. These results indicate the lack of stimulus by the relatively high Cu levels in the control and ZnO groups on MT levels. The inability of Cu to act as a stimulus for MT induction is well documented (Saylor et al., 1980; Peterson and Mercer, 1988). Since MT present in sheep is not stimulated by Cu, this may be one of the causes for their high susceptibility to Cu toxicosis. Furthermore, the limited capacity of sheep to synthesize MT in the intestinal mucosa (Saylor et al., 1980) may also be a factor. This limited ability to block Cu absorption at the intestinal level is supported by the high levels of Cu in sheep livers in all treatments.

Tissue Cu levels were not greatly affected by Zn supplementation. There was a decrease from 30 to 50% in muscle Cu concentrations for animals Zn supplemented compared to controls, however. Copper muscle concentrations of controls were probably due to one of two factors or both, the high dietary Cu levels, or the low dietary Zn levels of the control diet. A diet of 100 mg/kg Zn has been shown to decrease liver Cu storage (Pope, 1971). This decrease did not occur in this experiment and actually the mean Cu content for the control group was slightly lower (but not different) than that of any other group.

Metallothionein has been shown to bind Cu with a very high affinity. Therefore, Cu present in liver, kidney and pancreas of the ZnLys treated animals is stored more in a

complex with MT than the Cu present in any of the other treatments. In a similar manner, because of its relatively higher MT values, the liver, kidney and pancreas Cu in the ZnMet and ZnSO₄ treatments is bound to MT in comparison with ZnO and the negative control. Despite the higher MT levels in these tissues, this protein accounts for only a small portion of the Cu and Zn concentration in these tissues. The unexplainable factor is that the serum Cu levels were similar for all treatments and controls should have had higher serum Cu concentrations. This is expected since serum Cu (ceruloplasmin) is a hormonally regulated process. The only consolation is that levels for the control treatment were increasing (but not different) by the end of the experiment.

Very few studies have evaluated the actual biological value of different organic Zn sources. Results of this study indicate that the organic Zn sources can be more (ZnLys) or equally (ZnMet) available as the best inorganic Zn source. There were no differences in the particular target pools (or tissues other than liver, kidney and pancreas) for the Zn sources, suggesting that Zn from the different sources may be metabolized equally in those tissues. Zinc from the ZnLys supplementation may be metabolized differently than that of the other sources because of its higher levels in the liver, kidney and pancreas.

Implications

Organic sources of Zn (ZnLys and ZnMet) have equal or greater availability than the most available inorganic source (ZnSO_4) and may be metabolized differently in some tissues. It is suggested that the highly increased synthesis of MT is proof that ZnLys is a more bioavailable source of Zn. Research is needed to determine if Cu toxicity in sheep can be more effectively suppressed with the use of ZnLys.

Summary and Conclusions

A study was conducted to compare supplemental ZnLys, ZnMet, ZnSO_4 , and ZnO on Zn, Cu and MT concentrations in various fluids and tissues of 40 wether lambs. Supplemental Zn (360 mg/kg) was fed for 3 wks, withdrawn for 4 wks and then resumed for another wk. Mineral (Zn and Cu) concentrations were determined in serum, liver, pancreas, kidney, bone, bone marrow, hoof, and leg muscle, and only Zn was determined in skin and cornea. Metallothionein content was determined in liver, pancreas and kidney. By d 49 serum Zn had increased less ($P < .05$) for controls than all but ZnMet, and on d 55 it had increased more ($P < .05$) for ZnLys than all but ZnSO_4 . There were no treatment effects in serum Cu content, but overall Cu content fell slightly. The ZnLys treatment had the highest ($P < .05$) Zn accumulation (581, 389, and 340 mg/kg) for kidney, liver and pancreas, respectively. Both ZnSO_4 and ZnMet treatments had higher ($P < .05$) liver Zn concentrations

(195 and 198 mg/kg, respectively) than the control treatment (127 mg/kg). Mean Zn content of bone, bone marrow, cornea, skin, hoof and muscle was not different ($P > .05$) among treatments. The ZnLys treatment had the highest ($P < .05$) MT levels of 79, 167, and 68 μg MT/g for liver, kidney, and pancreas, respectively. Mean muscle Cu concentration was highest ($P < .05$) for controls (10 mg/kg). Organic sources of Zn have equal or greater availability than the most available inorganic source and may be metabolized differently in some tissues.

CHAPTER 5
DEVELOPMENT OF ACUTE COPPER POISONING IN
SHEEP FED ORGANIC OR INORGANIC COPPER

Introduction

It is well known that sheep are one of the most sensitive animals to Cu toxicosis. The exact biochemical etiology of the Cu toxicity is not well known. Saylor et al. (1980) suggested that because of the low capability for intestinal metallothionein (MT) synthesis by sheep, the Cu absorption process was not as well regulated as that of other species. Furthermore, it is well known that MT in the tissues of sheep do not respond to increased inorganic Cu levels (Saylor et al., 1980; Peterson and Mercer, 1988).

The toxicity, however, can be either chronic or acute depending on the dosage and time of exposure to the mineral. Sheep that are supplemented with relatively high doses of inorganic Cu during an extended period of time may die of hemolytic crisis. During the first phase of the increased dose (> 26 mg/d), the liver and other tissues of the sheep accumulate Cu. This phase may last from 6 to 10 weeks or longer. After the tissues are saturated with Cu, the blood levels of Cu begin to rise, the animals lose their appetite, develop an excessive thirst and become jaundiced. During this

hemolytic crisis, the liver becomes cirrhotic and the kidneys turn very dark, hemoglobin-stained. This crisis eventually leads to the death of the animal within a few days.

It has been reported that Cu from some organic sources may be more bioavailable than that from inorganic sources. It has also been postulated, however, that the supplementation of a complexed source of copper may retard or even prevent the onset of this toxicosis. Ishmael et al. (1977) proved that the supplementation of copper methionate in subcutaneous injections was not as toxic as Cu Ca EDTA. It was also hypothesized (Ashmead and Jeppsen, 1993) that the toxicity of complexed minerals is lower than salts due to sterically shielding the metals with the amino acids which have bent around the metals as a consequence of forming the bond. Spears et al. (1991) hypothesized that certain trace mineral chelates or complexes may enter different pools in the body than the inorganic forms. This fact alone may make the Cu from Cu lysine (CuLys) more available but not as toxic as CuSO_4 .

Because of the known information on Cu toxicosis, the tissues to analyze for Cu would include liver and kidney. Serum Cu and Zn and tissue Zn analyses would also be necessary because of the known interactions between these minerals.

The objective of this study was to compare toxicity of CuLys and CuSO_4 when supplemented at concentrations that would cause a chronic toxicity to sheep.

Materials and Methods

Three crossbred wethers and one crossbred ewe averaging 46 kg were randomly assigned to one of two groups. The two groups consisted of basal diet + 250 mg/kg of supplemental Cu from either CuSO₄ or CuLys. Lambs were housed in individual wooden pens (1.4 m²) with expanded metal floors in an open side barn. Feed intake was restricted to 1200 g/hd daily (as-fed basis) and tap water was available ad libitum. The diet (Table 5-1) was formulated to be adequate in protein, energy, vitamins, and minerals for this class of sheep (NRC, 1985). Lambs were fed the treatment diets for 4-11 wks

TABLE 5-1. Composition of basal diet offered to sheep (as fed)^a.

Ingredient	Percentage
Ground yellow corn	59
Cotton seed hulls	21
Soybean meal (44% CP)	12
Alfalfa meal (14% CP)	3
Corn oil	3
Trace mineral salt ^b	1
Ground limestone	1
Vitamins A and D ₃ ^c	.0008

^a Analysis indicated 290 and 303 mg Cu/kg of feed for CuSO₄ and CuLys treatments, respectively. Diets formulated to provide 250 mg supplemental Cu/kg diet.

^b Provided 2.4 mg of I (KI), .48 mg of Co (CoCO₃), 82 mg of Fe (FeSO₄), 48.19 mg of Mn (MnSO₄), 40 mg Zn (ZnSO₄) and no Cu per kg of diet.

^c Provided 5,000 IU of vitamin A and 500 IU of vitamin D₃ per kg of diet.

following a 7 d adjustment period during which all the animals were fed the basal diet. Protocol for animal care had been approved by the University of Florida's Institutional Animal Care and Use Committee.

Blood samples were obtained via jugular venipuncture on d 1 before animals were administered the concentrate and biweekly thereafter. To obtain serum, samples were centrifuged at 700 x g for 25 min, supernatant decanted and frozen until analyzed for Zn and Cu concentrations. To obtain hematocrit (HCT) percentages, blood was centrifuged at 700 x g for 10 min. Serum and blood samples were then submitted to a lab on the day of withdrawal for creatine kinase (CK), τ -glutamyl-transferase (GGT), aspartate amino transferase (AST) and heinz bodies determinations. At the end of the experiment on d 78, all surviving animals were stunned with a captive bolt shot and euthanized by exsanguination.

Parts of the liver and kidney were excised and taken to the veterinary hospital for necropsy and other parts were frozen for further mineral analyses. Copper and Zn in tissues, serum and diet samples were analyzed by air acetylene flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with AS-50.

Results

Animal 77 (CuSO_4) died of natural causes (related to Cu toxicosis) on d 28 and was consuming an average of only 20 g/d

for 14 d prior to death. Animal 46 (CuLys) was slaughtered on d 43 after it had only been eating 25 g/d for 5 d. Animal 1 (CuLys) was slaughtered on d 78 after it had only been eating 30 g/d for 7 d. The ewe lamb (288, CuSO₄) never showed anorexia or any other sign which reflected a Cu toxicity and was slaughtered with the last animal to end the experiment.

Overall, serum Cu concentrations rose sharply in all animals, except 288 (CuSO₄), shortly before death (Figure 5-1). For two of the animals (1, CuLys and 77, CuSO₄) there was a sharp decrease in serum Cu immediately before death. There were no plateaus in the serum Cu response curve of animal 77 which might indicate a decreased ability to control serum Cu concentration when compared to animals 1 and 46 (CuLys).

Overall, serum Zn (Figure 5-2) concentrations fluctuated greatly for all animals but were mostly above the .65 µg/ml suggested by McDowell et al. (1984) where a deficiency might be expected. It is also interesting that for the two CuLys treated sheep, serum Zn concentrations fell dramatically before death. The other animal (77) that exhibited signs of Cu toxicity showed low concentrations but also a rise right before death.

In general, blood HCT (Figure 5-3) started rising before death for three of the animals (1, 77 and 288). For the animal that died of natural causes, the blood HCT fell dramatically before death.

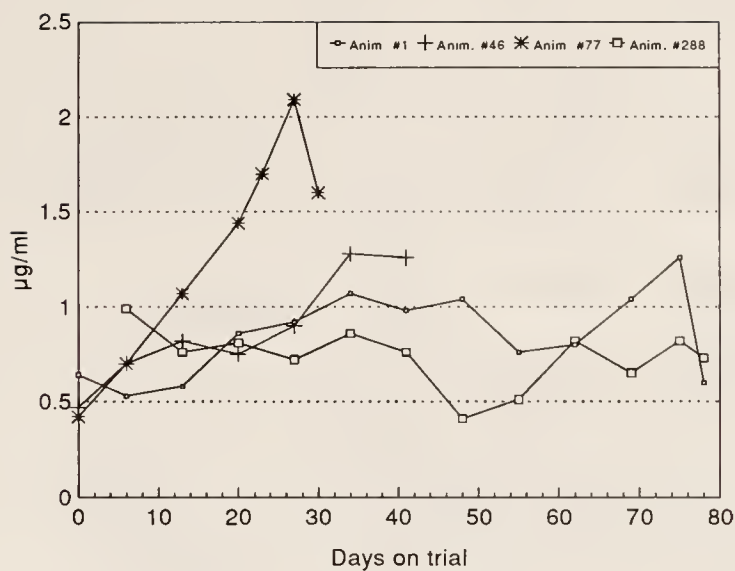


FIGURE 5-1. Serum Cu concentrations for sheep supplemented with toxic levels of CuSO_4 (77 and 288) and CuLys (1 and 46).

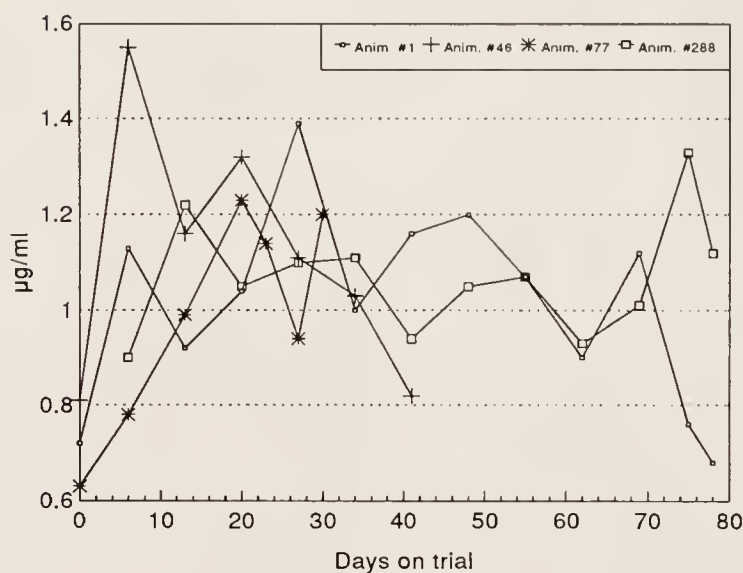


FIGURE 5-2. Serum Zn concentrations for sheep supplemented with toxic levels of CuSO_4 (77 and 288) and CuLys (1 and 46).

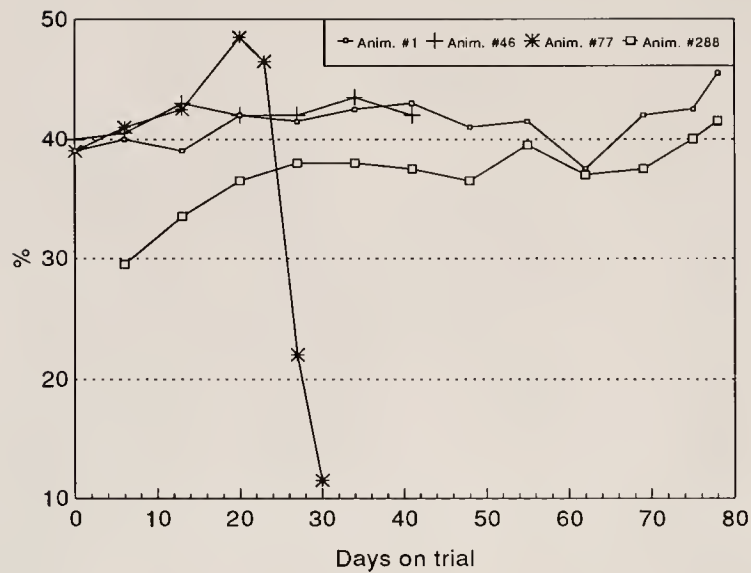


FIGURE 5-3. Blood hematocrit for sheep supplemented with toxic levels of CuSO_4 (77 and 288) and CuLys (1 and 46).

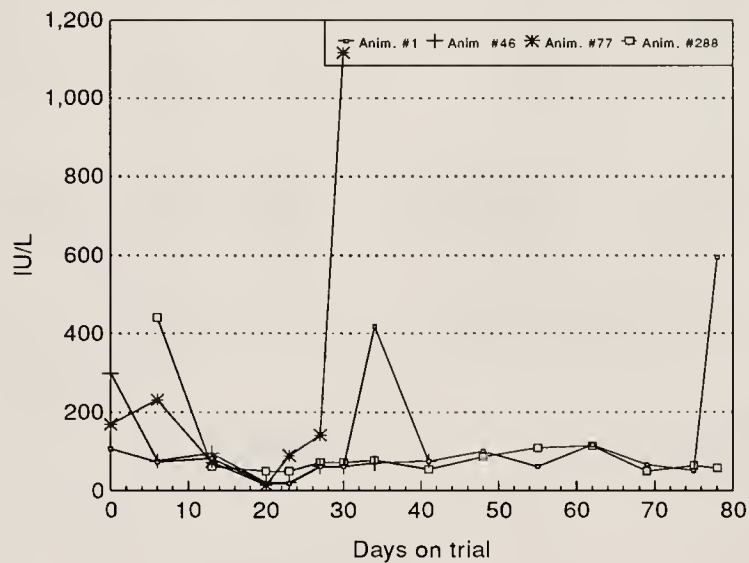


FIGURE 5-4. Serum creatine kinase concentrations for sheep supplemented with toxic levels of CuSO_4 (77 and 288) and CuLys (1 and 46).

Overall, serum CK (Figure 5-4) activity decreased with the beginning of Cu supplementation. Creatine kinase concentrations were highly increased for animals 1 and 77 which also showed increased serum Cu and HCT at the time of death. On the other hand animal 46 did not show such an increase in CK levels. There also a peak for animal 1 on d 34 but it went back to the previous concentration by the next sampling time.

Overall, GGT concentrations (Figure 5-5) rose in the beginning of the trial but fluctuated throughout for all the wether lambs. Concentrations of GGT rose for the two wethers receiving CuLys on d 27 and remained high until death. The other wether (77) had highly variable GGT level which was low at the time of death. The GGT level for the ewe was relatively constant throughout the experiment.

Overall, AST concentrations rose from the beginning of the experiment for all the wethers (Figure 5-6). These concentrations also decreased dramatically before death for two of the animals (1 and 77) and they remained high for the other (46).

Heinz bodies determination was positive only for animal 77 on d 27 with 41% noted. Another interesting detail was noticed in the blood serum. On d 20 and 23, blood serum for animal 77 had a mustard color to it and on d 27 it turned to red which was indicative of the full blown hemolytic crisis. The animal was found dead by d 30. Furthermore, on d 75,

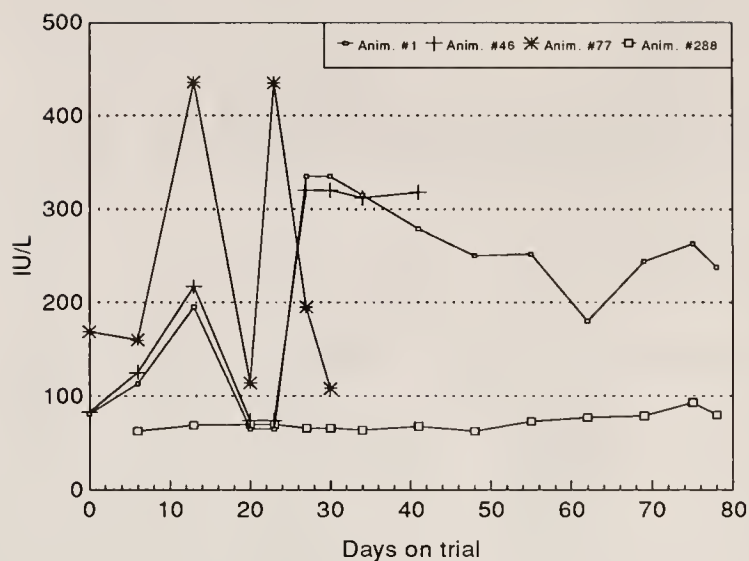


FIGURE 5-5. Serum γ -glutamyltransferase levels for sheep supplemented with toxic levels of CuSO_4 (77 and 288) and CuLys (1 and 46).

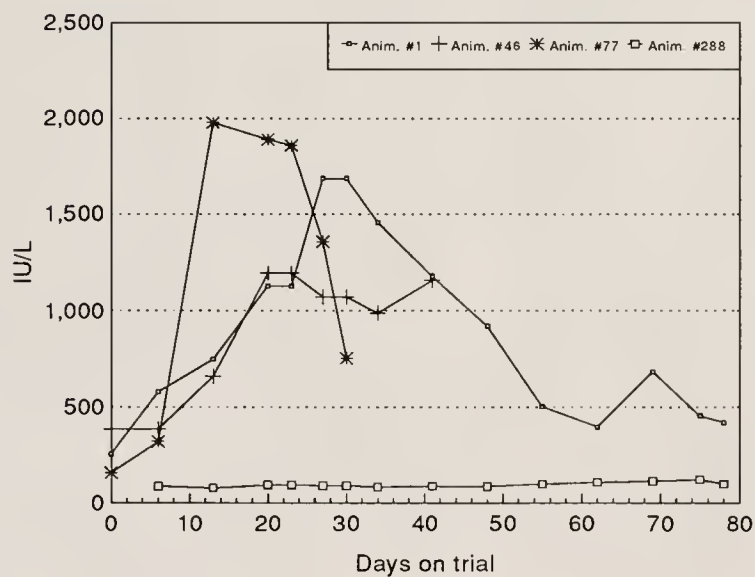


FIGURE 5-6. Serum aspartate amino transferase levels for sheep supplemented with toxic levels of CuSO_4 (77 and 288) and CuLys (1 and 46).

blood serum for animal 1 also exhibited this mustard color which turned to red on d 78. In contrast, animal 46 did not show any abnormal color changes.

Animal 1 had a very high liver Cu concentration (Table 5-1) which might have been expected since that animal had received the high Cu diet for 78 d. All other animals (46, 77 and 288) had relatively similar liver Cu levels. Kidney Cu levels varied with animal 1 having the highest (846 mg/kg) and animal 288 having the lowest (32 mg/kg).

Liver Zn concentrations (Table 5-2) were higher for animals 1 (CuLys) and 77 (CuSO₄), the animals suspected of developing a full-blown Cu toxicosis. Kidney Zn concentrations were very similar for all animals.

The necropsy report for animal 1 (CuLys), slaughtered on d 78, indicated a mild chronic multifocal bronchopneumonia with aspirated foreign material. Also, severe chronic cholangiohepatitis, severe chronic periportal

TABLE 5-1. Liver and kidney Cu concentrations of sheep exposed to high Cu levels^a.

Animal #	Liver (mg/kg)	Kidney (mg/kg)
1 (CuLys)	2552	846
46 (CuLys)	985	530
77 (CuSO ₄)	1036	426
288 (CuSO ₄)	937	32

^a Dry matter basis.

TABLE 5-2. Liver and kidney Zn concentrations of sheep exposed to high Cu levels^a.

Animal #	Liver (mg/kg)	Kidney (mg/kg)
1 (CuLys)	191	117
46 (CuLys)	90	123
77 (CuSO ₄)	168	126
288 (CuSO ₄)	107	122

^a Dry matter basis.

and centrilobular hepatocyte necrosis and histiocytic hyperplasia with megalocytosis, biliary hyperplasia and portal fibrosis were observed in the liver. In the kidney a mild chronic lymphocytic pyelonephritis with scattered tubular casts was reported. The absence of hemoglobinuric nephrosis suggested that the Cu accumulation in the liver was subclinical at the time of death.

The necropsy report for the liver of animal 46 (CuLys), slaughtered on d 41, showed an overall reduction of hepatocytes and increased fibrous stroma. There was also severe autolysis and biliary stasis. The report for the liver of animal 77 (CuSO₄), which died on d 30, showed a severe, diffuse hepatopathy with cytoplasmic Cu accumulation and individual hepatocyte necrosis. Also there was a severe, intrahepatic cholestasis and a mild, multifocal, suppurative, acute hepatitis. The pathological report for animal 288 (CuSO₄), slaughtered on d 78, indicated severe chronic multifocal dystrophic mineralization, fibrosis, and

granulomatous inflammation which was most likely due to aspirated material or metabolic injury to lung collagen. The liver showed a mild Kupffer cell hyperplasia.

Discussion

Serum Cu concentrations provide a good indicator of Cu status of ruminants. The liver and kidneys of animal 77 could not tolerate the dietary Cu as well as the others as indicated by the relatively low levels of Cu the liver and kidney and the high serum Cu at the time of death compared to animal 1.

Animal 46 may not have died solely from a Cu toxicosis. The factors which seem to substantiate this theory include the low CK levels, the high GGT and AST levels (no decline), the failure of HCT to rise, and the absence of hepatitis at the time of death compared to the animals which exhibited signs of Cu toxicosis.

Animal 288 was apparently never affected by Cu excess. The exact cause of this is not known because the animal had relatively similar liver Cu levels as the other animals but showed no change in any of the serum enzymes.

Blood HCT percentages show a slight increase in erythrocytes during the days prior to death which might reflect a decreased capacity to carry oxygen by the cells. A dramatic decrease in HCT was seen in animal 77 which represents the full onset of the hemolytic crisis. This decline was not seen with the other animals perhaps because

they were euthanized after anorexia had been seen for several days.

The CK analysis reflects myodegradation (Meyer et al., 1992) in animals 1 and 77 prior to death which might have been caused by the excess Cu in the blood being picked up by the muscle and rupturing the cells. The release of GGT from the hepatic cells of animal 77 was greater than that of all other animals. The increased GGT activity represents a cholestasis (Meyer et al., 1992) by d 13 for all Cu affected animals. It was interesting to see animals 46 and 1 survive longer with such high levels of serum GGT as compared to 77. The source of the high AST levels is the liver (Duncan and Prasse, 1977). A rapid increase occurred in animal 77 and a slower one for animals 1 and 46 which is proportional to the number of hepatocytes damaged. Animal 1 seems able to recover from the original cholestasis due to decreased AST levels.

The heinz bodies seen in animal 77 before death represents an increased hemoglobin turnover (Meyer et al., 1992) resulting from the hemolytic crisis. The yellow colored serum appearing before death may be an accumulation of bile acids and bilirubin (also due to hemolytic crisis) in peripheral circulation but these tests were not conducted. It is necessary to conduct another trial to determine MT levels of animals supplemented with CuLys to evaluate the relatively increased survivability of those animals.

Results of this study do not indicate CuLys to be more or less toxic than CuSO_4 . These data do indicate that there were some plateaus in the different serum enzyme and Cu concentrations for those animals receiving CuLys, which were not present with animal 77.

Implications

The use of high Cu supplementation in sheep, from this small scale study, and the development of toxicity is not dependant on the source used. The supplementation of CuLys as a source of "safe" Cu when supplemented in excess is inconclusive and needs to be further researched.

Summary and Conclusions

A study was conducted to compare toxicity of CuLys and CuSO_4 when supplemented at concentrations that would cause a chronic toxicity to sheep. Four animals, 3 crossbred wethers and 1 crossbred ewe averaging 46 kg were randomly assigned to 250 mg of supplemental Cu from either CuLys or CuSO_4 . Lambs were fed the treatment diets for 4-11 wks following a 7 d adjustment period. Blood samples were taken on d 1 before animals were administered the concentrate and biweekly thereafter. Serum and blood samples were analyzed for CK, GGT, AST, HCT, and heinz bodies. Sections of liver and kidney were excised and necropsied, and together with serum and diet were analyzed for Cu and Zn. The liver and kidneys of animal

77 (CuSO_4) could not tolerate the high dietary Cu and resulted in a rapid increase in serum Cu compared to others. Animal 288 was apparently never affected by Cu excess. Myodegradation was present in animals 1 and 77 prior to death and was probably caused by excess Cu in blood. The use of high Cu supplementation in sheep and the development of toxicity was not dependant on source used.

CHAPTER 6
INTERACTION OF DIFFERENT ORGANIC AND
INORGANIC ZINC AND COPPER SOURCES
FED TO RATS

Introduction

For many years it has been known that the amount of Zn absorbed can be influenced by the amount of dietary Cu (Miller et al., 1979). The absorption process of Zn and Cu is not completely understood. Perhaps the first site for interaction of Zn and Cu is the intestinal membrane. Subsequently, through the binding of these minerals to metallothionein (MT) an additional interaction could occur (Cousins and Hempe, 1990). This metalloprotein has a higher affinity for Cu than for Zn.

A recent study evaluated mineral content of rat tissues fed different levels of inorganic sources of Zn and Cu (Larsen and Sandstrom, 1992). There was a high interaction which affected not only the intestinal absorption, but also distribution of previously absorbed elements in tissues.

Several products offering minerals complexed with amino acid are available for mineral supplementation. In contrast to our knowledge with inorganic forms of Cu and Zn, it is not

known if Zn and Cu will interfere with each other if they are provided in complexed rather than inorganic forms.

The purpose of this study was to compare bioavailability, interactions and retention of complexed and inorganic sources of Zn and Cu fed to rats.

Materials and Methods

Sixty-three male Charles Sprague-Dawley (CD) strain rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 71.5 ± 7.3 g (mean \pm SEM) were individually housed in suspended, stainless steel cages in an environmentally controlled room with a 12-h light:dark cycle.

Rats were individually fed a purified diet and deionized water ad libitum. The purified diet (Research Diets, New Brunswick, NJ) was based on the AIN-76a formulation and contained the ingredients specified in Table 6-1. The purified diet contained .34 and .71 mg/kg of Zn and Cu, respectively. The diet was formulated to be adequate in protein, energy, vitamins, and minerals for this class of rats (NRC, 1978).

Different Zn (Zn methionine, ZnMet; Zn lysine, ZnLys; Zn sulfate, ZnSO_4) and Cu (Cu lysine, CuLys; Cu sulfate, CuSO_4 ; Cu oxide, CuO) sources were added to the basal diet at 30 mg/kg of Zn and 6 mg/kg of Cu to create a 3 X 3 factorial experiment (organic sources from Zinpro Corporation, Edina, MN; inorganic sources from Southeastern Minerals, Bainbridge,

TABLE 6-1. Composition of purified diet fed to rats (As-fed).^a

Ingredient	g/kg
Egg white solids	200
Cornstarch	150
Sucrose	503
Cellulose	50
Corn oil	50
AIN-76 mineral mix ^{b,c}	35
AIN-76 vitamin mix ^c	10
Biotin	<u>d</u>
Choline bitartrate	2

^a Zn and Cu analysis indicated (mean \pm SD) of 29 ± 2 mg Zn/kg and $5.3 \pm .5$ mg Cu/kg of all supplemented diets.

^b Contains no Zn or Cu.

^c Contents of the mineral and vitamin mix are specified in appendix Tables A-1 and A-2, respectively.

^d Provided .004 g biotin/kg.

GA). Seven rats were randomly assigned to each of these treatments.

Supplemented diets were fed for 4 wks at which point four randomly selected rats from each treatment were sacrificed (first phase). The rest of the animals were fed the basal diet (Table 6-1) for an additional week (second phase) and then sacrificed. Protocol for animal care had been approved by the University of Florida's Institutional Animal Care and Use Committee. All rats were anesthetized by inhaling MetafaneTM (Methoxyflurane) and bled by cardiac puncture.

To obtain heparinized plasma, blood was centrifuged at 700 x g for 25 min, supernatant decanted and frozen until analyzed for Zn and Cu. Tissues were immediately excised. The liver and both kidneys were frozen at - 80°C, and the rear leg muscles (biceps femoris, vastus lateralis, and gluteous, combined) and bones (femur, tibia, and fibula, combined) were frozen at - 20°C.

Total MT was measured in kidney and liver by the $^{109}\text{Cd}^{2+}$ -binding method (Eaton and Toal, 1982). The Zn and Cu concentrations in plasma, liver, kidney, muscle and bone were measured by air acetylene flame atomic absorption spectrophotometry on a Perkin-Elmer Model 5000 with AS-50.

All data was analyzed using SAS (SAS, 1988). Tissue and plasma Zn, Cu and MT data were analyzed using GLM procedure and in case of significance ($P < .05$) Waller-Duncan's K-ratio T test was used for multiple comparisons (Waller and Duncan, 1969).

Results

Weight gains were not different ($P > .05$) among treatments or experimental phases, but there was a tendency ($P = .07$) for CuLys to have a higher ADG than CuSO_4 for phase 1. Diet intakes were similar for all groups for both phases with no anorexia noted. When not mentioned there were no interaction effects ($P > .05$).

Phase 1

Plasma Zn concentrations of rats were not affected ($P > .05$) by Zn or Cu source (Table 6-2). Plasma Cu

TABLE 6-2. Mean plasma Zn and Cu concentrations for rats supplemented with different sources of Zn and Cu ($\mu\text{g/ml}$)^a.

Sources	Zn	Cu
ZnSO ₄	2.5	1.0
ZnMet	2.2	.8
ZnLys	2.6	.9
CuO	2.2	.2 ^b
CuSO ₄	2.5	1.2 ^c
CuLys	2.6	1.3 ^c

^a SEM are as follows: Zn = .75, Cu = .27.

^{b,c} Means with different subscripts within column and mineral differ ($P < .05$).

concentrations, on the other hand, were lower ($P < .05$) for CuO than CuSO₄ or CuLys supplemented rats.

There were no main effects (Zn or Cu; $P > .05$) for the Zn concentrations for most tissues (Table 6-3). Mean Zn concentrations were relatively constant for all tissues across treatments. Bone Zn concentrations, however, were higher ($P < .05$) for CuLys than for CuO supplemented rats.

There was an interaction effect for bone Zn concentrations. Bone Zn concentrations were higher ($P < .05$) for CuLys than CuSO₄ rats that were supplemented with ZnSO₄ and CuLys supplementation resulted in higher ($P < .05$) bone Zn

concentrations than did CuO for rats receiving ZnMet (Figure 6-1A). There were no bone Zn differences ($P > .05$) from Cu source for the ZnLys source. Bone Zn concentrations were

TABLE 6-3. Mean tissue Zn concentrations for rats supplemented with different sources of Zn and Cu (mg/kg, DMB^a)^b.

Sources	Bone	Kidney	Liver	Muscle
ZnSO ₄	149	84	76	58
ZnMet	150	85	77	57
ZnLys	150	84	76	58
CuO	147 ^c	85	75	56
CuSO ₄	150 ^{c,d}	85	77	58
CuLys	153 ^d	83	77	58

^a DMB= Dry matter basis, bone also fat free.

^b SEM are as follows: bone = 6, kidney = 14, liver = 10, muscle = 8.

^{c,d} Means with different subscripts within column and mineral differ ($P < .05$).

higher ($P < .05$) for ZnLys than ZnSO₄ rats that received CuSO₄ supplementation, however, ZnLys had the lowest ($P < .05$) bone Zn concentrations when CuLys was the Cu supplementation source (Figure 6-1B). There were no differences ($P > .05$) in Zn source for Zn tissue levels when CuO was the supplemental Cu source.

All tissue Cu concentrations were affected ($P < .05$) by supplemental Cu source (Table 6-4). In all tissues where Cu was measured, CuO was the lowest ($P < .05$) available source of Cu. Furthermore, CuSO₄ supplemented rats had higher ($P < .05$) Cu concentrations in muscle than from CuLys supplementation.

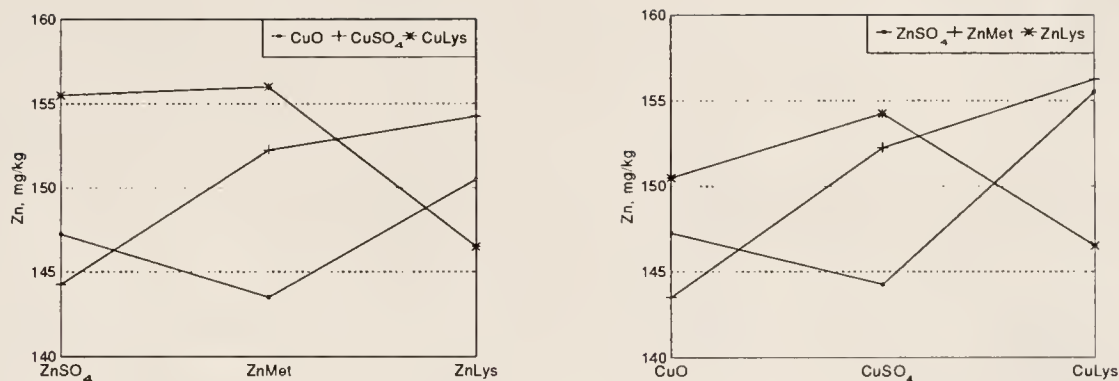


FIGURE 6-1 A+B. Mean bone (dry, fat free basis) Zn concentrations for rats supplemented with different sources. A) (Left) Different Cu sources when supplementing different Zn sources. B) (Right) Different Zn sources when supplementing different Cu sources. SEM (mg/kg) 6.0.

TABLE 6-4. Mean tissue Cu concentrations for rats supplemented with different sources of Zn and Cu (mg/kg, DMB^a)^b.

Sources	Kidney	Liver	Muscle
ZnSO ₄	27	11	7
ZnMet	29	12	6
ZnLys	30	12	6
CuO	22 ^c	9 ^c	4 ^c
CuSO ₄	32 ^d	13 ^d	8 ^d
CuLys	32 ^d	14 ^d	6 ^e

^a DMB= Dry matter basis.

^b SEM are as follows: kidney = 6, liver = 2, muscle = 2.

^{c,d,e} Means with different subscripts within column and mineral differ (P < .05).

Different Zn sources did not affect ($P > .05$) tissue Cu contents.

Kidney MT content followed the same pattern as Cu concentrations with CuO being the lowest ($P < .05$) MT inducer (Table 6-5). There was no effect ($P > .05$) of Zn source on tissue MT contents for any tissue and no Cu effect ($P > .05$) for liver MT.

TABLE 6-5. Mean tissue metallothionein concentrations for rats supplemented with different sources of Zn and Cu ($\mu\text{g MT/g}^{\text{a}})^{\text{b}}$.

Sources	Kidney	Liver
ZnSO ₄	77	40
ZnMet	76	42
ZnLys	80	38
CuO	68 ^c	46
CuSO ₄	82 ^d	38
CuLys	84 ^d	37

^a $\mu\text{g MT/g} = \mu\text{g of Metallothionein per gram of wet tissue.}$

^b SEM are as follows: kidney = 10, liver = 12.

^{c,d} Means with different subscripts within column and mineral differ ($P < .05$).

Phase 2

Plasma Zn concentrations of depleted rats were not affected ($P > .05$) by Zn or Cu source (Table 6-6). Plasma Cu concentrations of depleted rats, on the other hand, were lower ($P < .05$) for CuO than CuLys supplemented rats.

There were no main effects (Zn or Cu; $P > .05$) for the Zn concentrations for most tissues of depleted rats (Table 6-7).

TABLE 6-6. Mean plasma Zn and Cu concentrations for rats supplemented with different sources of Zn and Cu for 4 wks and then depleted for 1 wk ($\mu\text{g/ml}$)^a.

Sources	Zn	Cu
ZnSO ₄	1.7	.2
ZnMet	2.0	.4
ZnLys	1.6	.3
CuO	1.8	.1 ^b
CuSO ₄	1.7	.2 ^{b,c}
CuLys	1.8	.5 ^c

^a SEM are as follows: Zn = .34, Cu = .22.

^{b,c} Means with different subscripts within column and mineral differ ($P < .05$).

TABLE 6-7. Mean tissue Zn concentrations for rats supplemented with different sources of Zn and Cu for 4 wks and depleted for 1 wk (mg/kg, DMB^a)^b.

Sources	Bone	Kidney	Liver	Muscle
ZnSO ₄	153	68	66	53
ZnMet	155	68	69	55
ZnLys	157	62	70	57
CuO	153	71 ^c	67	56
CuSO ₄	158	59 ^d	71	55
CuLys	154	67 ^{c,d}	67	54

^a DMB= Dry matter basis, bone also fat free.

^b SEM are as follows: bone = 5, kidney = 9, liver = 5, muscle = 8.

^{c,d} Means with different subscripts within column and mineral differ ($P < .05$).

Kidney Zn concentrations were lower ($P < .05$) resulting from CuSO_4 supplementation than for CuO supplemented rats.

There was an interaction effect for kidney Zn concentrations after depletion. Kidney Zn concentrations after depletion were highest ($P < .05$) for CuO and lowest ($P < .05$) for CuSO_4 supplementation in the rats also receiving ZnLys supplementation (Figure 6-2A). There were no kidney Zn differences ($P > .05$) from different Cu source for the ZnMet or ZnSO_4 supplemented rats. Kidney Zn concentrations were higher ($P < .05$) for ZnLys than ZnSO_4 supplemented rats that were also given CuO supplementation, however, ZnLys had the lowest ($P < .05$) kidney Zn concentrations when CuSO_4 was the Cu source (Figure 6-2B). There were no differences ($P > .05$) in Zn source when CuLys was the Cu source.

Most tissue Cu concentrations after depletion were not affected ($P < .05$) by supplemental Cu source (Table 6-8). In liver, however, CuO supplemented rats had the lowest ($P < .05$) Cu concentration. Different Zn sources did not affect ($P > .05$) tissue Cu contents.

There was no effect ($P > .05$) of Zn or Cu source on tissue MT contents for any tissue after 1 wk of depletion (Table 6-9).

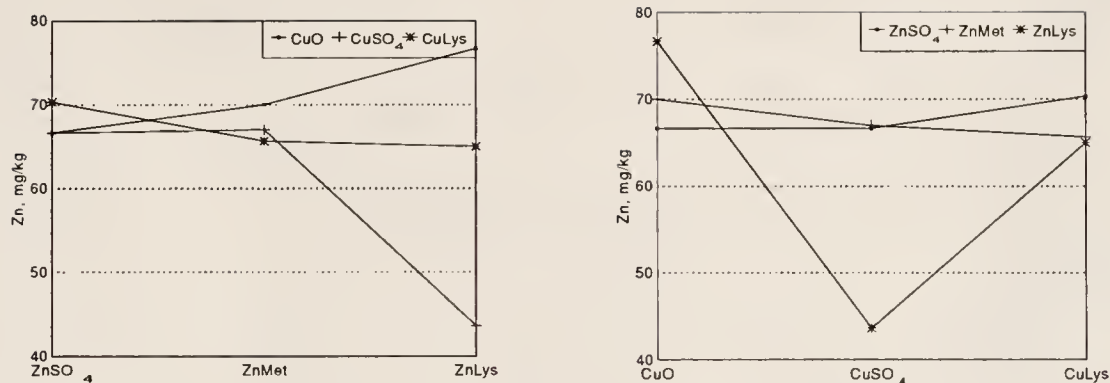


FIGURE 6-2 A+B. Mean kidney (dry basis) Zn concentrations for rats supplemented with different sources. A) (Left) Different Cu sources when supplementing different Zn sources. B) (Right) Different Zn sources when supplementing different Cu sources. SEM (mg/kg) 6.0.

TABLE 6-8. Mean tissue Cu concentrations for rats supplemented with different sources of Zn and Cu for 4 wks and then depleted for 1 wk. (mg/kg, DMB^a)^b.

Sources	Kidney	Liver	Muscle
ZnSO ₄	23	8	6
ZnMet	26	9	5
ZnLys	20	10	7
CuO	22	7 ^c	5
CuSO ₄	24	10 ^d	6
CuLys	23	11 ^d	7

^a DMB= Dry matter basis.

^b SEM are as follows: kidney = 8, liver = 2, muscle = 2.

^{c,d} Means with different subscripts within column mineral differ (P < .05).

TABLE 6-9. Mean tissue metallothionein concentrations for rats supplemented with different sources of Zn and Cu for 4 wks and then depleted for 1 wk ($\mu\text{g MT/g}^{\text{a}}$)^b.

Sources	Kidney	Liver
ZnSO ₄	44	30
ZnMet	37	32
ZnLys	42	30
CuO	43	32
CuSO ₄	40	33
CuLys	39	27

^a $\mu\text{g MT/g} = \mu\text{g}$ of Metallothionein per gram of wet tissue.

^b SEM are as follows: kidney = 9, liver = 6, no differences ($P > .05$).

Discussion

Plasma Zn and Cu concentrations for phase 1 of the experiment were very similar to those noted by Blalock et al. (1988) when inorganic Zn and Cu supplementation was used at different levels. Plasma Zn concentrations may have been slightly high in our study because of limited hemolysis of a few samples. The lower serum Cu concentration for animals supplemented with CuO confirms the poor bioavailability of this source (Kincaid, 1988). Plasma Zn and Cu concentrations decreased during the week of depletion. Following a similar pattern, CuLys supplemented rats had higher plasma Cu concentration than CuO supplemented rats but this time CuO was not different than CuSO₄ which could suggest a higher retention for CuLys.

Since tissue Zn concentrations were not affected by Zn source, this may suggest equal metabolism of all Zn sources in those tissues at this level of supplementation. Copper, however, may be involved in bone Zn deposition since CuO treated rats, which was less available, had lower bone Zn than CuLys treated rats. Most tissue Zn concentrations decreased during depletion, but bone showed no change. Kidney Zn concentrations of CuO supplemented rats were inexplicably higher than those of CuSO₄. Zinc retention based on tissue data for the different treatments was not affected.

Mean tissue Cu concentrations reflected the same trends as plasma concentrations indicating that CuO was less bioavailable than CuLys or CuSO₄. Furthermore, CuSO₄ supplemented rats had the highest muscle Cu concentrations which suggests that Cu from this source is taken up by muscle cells more readily. The only tissue to retain the same proportions of Cu following depletion to those before the beginning of depletion was the liver, as liver Cu concentrations of CuO supplemented rats were lower than other treatments. Kidney and muscle Cu concentrations, however, stabilized and there was no difference for the different sources, suggesting a lower retention for CuLys and CuSO₄. Copper retention in those tissues, represented by the lack of difference among sources, might also be influenced because Cu levels were marginal for rat tissues treated with CuO which had low plasma Cu concentrations.

The interaction effects shown in bone following supplementation indicate increased bone Zn deposition by the ZnLys and CuLys treatments except when combined, in which case bone Zn concentrations drop. This observation might support the theory that when complexed, the mineral is "smuggled" across the membrane by the other molecule's (in this case lysine) transport mechanism (Ashmead and Jeppsen, 1993). This also seemed to be the case when the sulfate forms were administered together. Copper has been suggested to have a role in the mineralization of growing bone, either in a cuproenzyme with ascorbate oxidase activity, or in its soluble ionic form (Allen and Solomons, 1984). It was interesting that this situation was not noted with other tissues but this is perhaps due to the fact that regular mineral transport mechanisms may not have been saturated. Following depletion, there was also an interaction effect, this time in kidney Zn concentrations. When ZnLys was the Zn source, CuO treated rats had the highest kidney Zn concentrations and CuSO₄ the lowest, which is puzzling.

Mean MT concentrations were not affected by Zn source suggesting equal biological values. They were, however, influenced by Cu source as CuO supplemented rats had lower MT concentrations. The levels shown in this study following supplementation were higher and following depletion were similar to those reported for kidney MT (42 ± 3 μ g MT/g) by Blalock et al. (1988) following a 14 d supplementation period

of equal levels of Zn and Cu. Liver levels reported in the same study ($42 \pm 3 \mu\text{g MT/g}$) were similar to those of our supplementation phase but higher than those of depleted rats. Furthermore, dietary Cu level was reported (Blalock et al., 1988) not to affect kidney MT concentration. The present study, the lowest available Cu source showed the lowest MT concentration, suggesting that Cu does influence MT expression when the available dietary Cu is very low. Following the week of depletion all MT levels stabilized and no differences were observed for different treatments. These results indicate that, at adequate supplemental levels, organic sources of Zn and Cu are metabolized similarly in most aspects as the best inorganic sources (CuSO_4 and ZnSO_4).

Implications

When supplementing adequate dietary levels of Zn and Cu (30 and 6 mg/kg, respectively), CuO is less available than CuLys and CuSO_4 , however under the conditions of this experiment, organic (ZnMet and ZnLys) and inorganic (ZnSO_4) sources of Zn were similar in bioavailability.

Summary and Conclusions

A study was conducted to compare bioavailability, interactions and retention of different sources of Zn and Cu fed to rats. Sixty-three male CD rats were fed individually a purified diet and deionized water ad libitum. The nine

treatments included were all combinations of three Zn (ZnMet, ZnLys, ZnSO₄) and three Cu (CuLys, CuSO₄, CuO) sources added to the basal diet at 30 mg/kg of Zn and 6 mg/kg of Cu forming a 3 X 3 factorial experiment. After the 4 wk supplementation phase, 4 randomly selected rats from each treatment were sacrificed (Phase 1). The remaining rats were fed the purified, unsupplemented diet for an additional week (Phase 2) and then sacrificed. Mineral (Zn and Cu) concentrations were determined in plasma, liver, kidney, bone, and muscle and MT content was determined in liver, and kidney. Plasma Cu concentrations were lower ($P < .05$) for CuO than CuSO₄ and CuLys supplemented rats. Bone Zn concentrations were higher ($P < .05$) for CuLys than for CuO supplemented rats. In all tissues where Cu was measured, CuO was the lowest ($P < .05$) available source of Cu. Furthermore, in muscle, CuSO₄ supplemented rats had higher ($P < .05$) Cu concentrations than CuLys. Kidney MT content followed the same pattern as Cu concentrations with CuO fed rats having the lowest ($P < .05$) MT concentrations. Plasma Cu concentrations of depleted rats were lower ($P < .05$) for CuO than CuLys supplemented rats. Kidney Zn concentrations were lower ($P < .05$) for CuSO₄ than for CuO supplemented rats after depletion. In liver, CuO supplemented rats had the lowest ($P < .05$) Cu concentration. Copper oxide was less available than CuLys and CuSO₄ when added in adequate dietary levels, however, organic (ZnMet and ZnLys) and inorganic (ZnSO₄) sources of Zn were similar.

CHAPTER 7 GENERAL SUMMARY AND CONCLUSIONS

Four experiments were conducted to compare the biological availability of different organic and inorganic sources of Zn and Cu by determining the Zn, Cu and metallothionein (MT) concentration of various fluids and tissues.

Experiment 1 was a 12 wk experiment conducted to compare supplemental ZnMet, ZnSO₄, and ZnO on Zn, Cu and MT concentrations in various fluids and tissues of 32 yearling beef cattle. Supplemental Zn (360 mg/d) was fed for 4 wks, withdrawn for 4 wks and then resumed for another 4 wks. Mineral (Zn and Cu) concentrations were determined in serum, liver, pancreas, kidney, bone, bone marrow, hair, hoof, and neck muscle and only Zn was determined in erythrocytes, skin and cornea. Metallothionein levels were determined in liver, pancreas and kidney. There were no treatment differences ($P > .05$) in serum or erythrocyte Zn content. Serum Cu concentrations tended to decrease with all treatments. There were no treatment differences ($P > .05$) in Zn and Cu tissue concentrations and liver, kidney and pancreas MT concentrations. Tissue Cu concentrations did not decline in

the supplemented treatments when compared to controls. It was concluded that, at adequate levels of dietary Zn, bioavailability of supplemental Zn source may be of less importance than when added to low Zn diets or at higher supplemental levels.

Experiment 2 was conducted to compare supplemental ZnLys, ZnMet, ZnSO₄, and ZnO on Zn, Cu and MT concentrations in various fluids and tissues of 40 wether lambs. Supplemental Zn (360 mg/kg) was fed for 3 wks, withdrawn for 4 wks and then resumed for another wk. Mineral (Zn and Cu) concentrations were determined in serum, liver, pancreas, kidney, bone, bone marrow, hoof, and leg muscle and only Zn was determined in skin and cornea. Metallothionein content was determined in liver, pancreas and kidney. By d 49 serum Zn had increased less ($P < .05$) for controls than all but ZnMet, and by d 55 had increased more ($P < .05$) for ZnLys than all but ZnSO₄. There were no treatment effects in serum Cu content, but overall Cu content fell slightly. The ZnLys treatment produced the highest ($P < .05$) Zn accumulation in kidney, liver and pancreas. Both ZnSO₄ and ZnMet treatments produced higher ($P < .05$) liver Zn concentrations than the control treatment. Mean Zn content of bone, bone marrow, cornea, skin, hoof and muscle was not different ($P > .05$) among treatments. The ZnLys treatment produced the highest ($P < .05$) MT levels for liver, kidney, and pancreas. Mean muscle Cu concentration was highest ($P < .05$) for controls (10

mg/kg). For this experiment with sheep, organic sources of Zn were of equal availability or more available than the most available inorganic source and may be metabolized differently in some tissues.

Experiment 3 was conducted to compare toxicity of CuLys and CuSO₄ when supplemented at concentrations that would cause a chronic toxicity to sheep. Three crossbred wethers and 1 crossbred ewe averaging 46 kg were randomly assigned to 250 mg of supplemental Cu from either CuLys or CuSO₄. Lambs were fed the treatment diets for 4-11 wks following a 7 d adjustment period. Blood samples were taken on d 1 before treatment began and biweekly thereafter. Serum and blood samples were analyzed for CK, GGT, AST, and heinz bodies. Sections of liver and kidney were excised and autopsied, and together with serum and diet analyzed for Cu and Zn. The liver and kidneys of one of the CuSO₄ treated sheep could not tolerate the high dietary Cu as did the others, resulting in a rapid elevation of serum Cu. The other CuSO₄ treated sheep was apparently never affected by Cu excess. Myodegradation was present in one animal from each treatment prior to death probably caused by excess Cu in blood and muscle. The use of high Cu supplementation in sheep and the development of toxicity from this limited number of animals was not dependant on source used.

Experiment 4 was divided into two phases. Phase 1 was conducted to compare bioavailability of different sources of

Zn and Cu by evaluating Zn, Cu and MT concentrations of selected tissues and plasma, and if interaction can be reduced by use of different sources. Phase 2 was conducted to compare Cu and Zn retention time after termination of supplementation. Sixty-three male CD rats were randomly assigned to one of nine treatments which were all combinations of three Zn (ZnMet, ZnLys, ZnSO₄) and three Cu (CuLys, CuSO₄, CuO) sources added to the basal diet at 30 mg/kg of Zn and 6 mg/kg of Cu, to create a 3 X 3 factorial experiment. After the 4 wk supplementation phase, 4 randomly selected rats from each treatment were sacrificed (Phase 1). The remaining rats were fed the purified, unsupplemented diet for an additional week (Phase 2). Mineral (Zn and Cu) concentrations were determined in plasma, liver, kidney, bone, and muscle and MT content was determined in liver, and kidney. Plasma Cu concentrations were lower ($P < .05$) for CuO than CuSO₄ and CuLys supplemented rats. Bone Zn concentrations were higher ($P < .05$) for CuLys than for CuO supplemented rats. In all tissues where Cu was measured, CuO was the lowest ($P < .05$) available source of Cu. Furthermore, in muscle, CuSO₄ supplemented rats had higher ($P < .05$) Cu concentrations than CuLys. Kidney MT content followed the same pattern as Cu concentrations with CuO producing the lowest ($P < .05$) MT concentrations. Plasma Cu concentrations of depleted rats were lower ($P < .05$) for CuO than CuLys supplemented rats. Kidney Zn concentrations were lower ($P < .05$) for CuSO₄ than for CuO supplemented rats after

depletion. In liver, CuO supplemented rats had the lowest ($P < .05$) Cu concentration. Copper oxide was less available than CuLys and CuSO_4 when added in adequate dietary levels, however, organic (ZnMet and ZnLys) and inorganic (ZnSO_4) sources of Zn were similar.

APPENDIX

TABLE A-1. AIN-76A mineral mix without added Zn or Cu (As-fed)^a.

Ingredient	g	g/35 g
Calcium Phosphate, Dibasic (29.5% Ca, 22.8% P)	500	Ca 5.2 P 4
Magnesium Oxide (60.3% Mg)	24	Mg .5
Potassium Citrate, 1 H ₂ O (36.2% K)	220	K 3.6
Potassium Sulfate (44.9% K, 18.4% S)	52	S .33
Sodium Chloride (39.3% Na, 60.7% Cl)	74	Na 1
		<u>mg/35 g</u>
Chromium Potassium Sulfate, 12 H ₂ O (10.4% Cr)	.55	Cr 2
Potassium Iodate (59.3% I)	.01	I .2
Ferric Citrate (21.2% Fe)	6	Fe 45
Manganous Carbonate (47.8% Mn)	3.5	Mn 59
Sodium Selenite (45.7% Se)	.01	Se .16
Sucrose	119.93	
Total	1000	

^a Mineral mix used in rat experiment, Chapter 6.

TABLE A-2. AIN-76A vitamin mix (As-fed)^a.

Ingredient	g	IU/10 g
Vitamin A Palmitate (500,000 IU/g)	.8	4,000
Vitamin D ₃ (100,000 IU/g)	1	1,000
Vitamin E Acetate (500 IU/g)	10	50
		<u>mg/10 g</u>
Menadione sodium Bisulfite (62.5% Menadione)	.08	.5
Biotin (1.0%)	2	.2
Folic Acid	.2	2
Nicotinic Acid	3	30
Calcium Pantothenate	1.6	16
Pyridoxine-HCl	.7	7
Riboflavin	.6	6
Thiamin HCl	.6	6
		<u>ug/10 g</u>
Cyanocobalamin (.1%)	1	10
Sucrose	978.42	
Total	1000	

^a Vitamin used in rat experiment, Chapter 6.

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BIOGRAPHICAL SKETCH

Luis Xavier Rojas was born on September 16, 1968, in Falls Church, Virginia, U. S. A. He then moved to Caracas, Venezuela. He attended high school at Institutos Educacionales Asociados in Caracas until the 10th grade. He went on to finish high school at Kents Hill School in Kents Hill, Maine, in order to learn the English language and graduated in May, 1985. He entered the University of Tennessee at Martin in September, 1985, and was awarded the degree of Bachelor of Science in Agriculture in May, 1989.

In August, 1989, he entered the University of Florida as a postbaccalaureate student. In January, 1990, he was accepted to the Graduate School at the University of Florida and began studies in the area of animal nutrition. He received the degree of Master of Science in animal science in August 1992. His thesis work was focused on the mineral status of Venezuelan cattle. During the same year he was awarded a teaching assistantship and began studies leading to the degree of Doctor of Philosophy at the University of Florida. At present he is a candidate to receive a Ph.D. in animal science in May 1994. In the future he plans to be a member of the faculty at La Universidad de Oriente in Maturin, Venezuela and continue working in the same area of study.

He has helped to teach part of the animal nutrition class. He is a member of Gamma Sigma Delta honor society of agriculture, and has been nominated Sigma Xi honor scientific society and to Alpha Zeta honor society.

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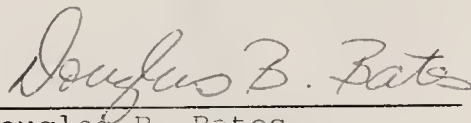
L. R. McDowell, Chair
Professor of Animal Science

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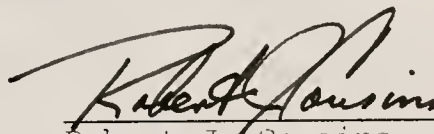
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